

Basic Slag as a Liming Material to Ameliorate Soil Acidity in Alfisols of Sub-tropical India

J.A. Bhat, Biswapati Mandal and G.C. Hazra

Department of Agricultural Chemistry and Soil Science,
Bidhan Chandra Krishi Viswavidyalaya, Mohanpur - 741252, Nadia, West Bengal, India

Abstract: Crop production on acid soils can be improved greatly by adjusting the pH to near neutrality. While soil acidity is commonly corrected by calcite, there is evidence that use of basic slag as an amendment can increase the pH of acid soils. The effect of calcite and basic slag (CaSiO_3) with different doses on soil acidity, nutrient availability and grain yield was determined in the experiments. Fourteen field experiments were conducted during the rabi season of 2003-2004 and 2004-2005 in Alfisols of Midnapur West and Purulia districts of West Bengal, INDIA. Besides liming materials, locally available organic resources e.g. farmyard manure (FYM) and poultry manure (PM) were also used along with basic slag to increase its efficacy. The treatments used were as follows: No lime, 1/5th LR (basic slag), 1/5th LR (calcite), 1/10th LR (basic slag), 1/10th LR (calcite), 1/5th LR (basic slag + FYM @ 5t/ha) and 1/5th LR (basic slag + PM @ 3t/ha). Results showed that both calcite and basic slag increased the grain yield of wheat. They were effective when applied @ 1/5th LR dose than 1/10th LR. On an average, calcite and basic slag caused an increase in grain yield to the extent of 21.9 and 31.0% over the no lime treatment, respectively. Results also showed that increase in the yield of wheat was more with basic slag 1/5th LR than with calcite. Incorporation of organic sources of nutrients particularly FYM and PM caused a further increase in yield, the magnitude being 56.2 and 60.2% respectively over the no lime treatment. Results of straw yield also showed the similar trend of change. Uptake of N and P by wheat plants showed that liming caused significant increases in their uptake. There was no significant increase in concentration of K with lime application. Organic matter addition enhanced the uptake of the nutrient elements viz., N, P and K. Results of the analysis of residual soil showed that total acidity, exchange acidity and hydrolytic acidity recorded a decrease upon liming.

Key words: Basic slag % calcite % liming acid soils % organic manures and wheat crop

INTRODUCTION

Soil acidity is a major factor limiting crop yield in vast areas of the world [1]. Acid soils occupy about 3.95 billion ha and account for 30% of the world's ice-free land area [2]. Soil acidity is particularly prevalent in the humid tropics and subtropics, climatic zones that encompass many of the countries struggling most to achieve self-sufficiency in food production. Out of the 328 million hectares of geographical area of India nearly 145 million hectares is cultivated and a rough estimate indicates that 48 million ha of soil is acidic in nature of which 25 m ha shows pH below 5.5 while about 23 m ha has pH between 5.6 and 6.5 [3].

Soil acidity is the major problem of the Alfisols of West Bengal, leading to severe toxicity of iron, aluminium and manganese accompanied by deficiency of phosphorus and low microbial activity leading to poor yield of crops [4]. In general fertility status of these soils is very poor and under strongly to moderately acidic soils the plant growth and development affect to a great extent. The crops grown on these problematic soils do not give remunerative return rather it lowers down the yield to a great extent. Because of the limited land resource it needs judicious management practice so that the yield of the different crops can be increased. So, one of the most important and particularly feasible management practices is the use of lime and liming materials to ameliorate the soil

acidity. The addition of lime raises the soil pH, thereby eliminating most major problems of the acid soils. Application of lime eliminates actual and exchange acidities, minimizes hydrolytic acidity, raises the calcium content in the soil [5]. Reduced soil acidity following liming also increases the availability of the several plant nutrients, notably phosphorus. Only about 20% of fertilizer phosphorus is taken up by the crop in the year of application. The remainder is fixed in the soil in various degrees of availability to succeeding crops. Therefore, one of the benefits of liming acid soils is the increased utilization of residual fertilizer phosphorus by crop. Liming creates a suitable environment (pH 6.0 - 6.5) for nitrifying bacteria, increase in aerobic N fixation process and organic matter decomposition process. Liming also enhances the mineralization of organic matter, thereby releasing inorganic plant nutrients such as N, P and S to soil solution.

Various liming materials are used to neutralise the soil acidity, thereby overcoming the problems associated with the acidification. One of the important liming materials is basic slag. Basic slag is a by-product of the basic open-hearth method of making steel and its neutralizing value is 86. The calcium contained is in the form of calcium silicate and reacts with soil acids in a manner similar to ground limestone. It also contains P_2O_5 ranging from 2-6% and some micronutrients and magnesium. Generally calcite ($CaCO_3$) is used as agricultural lime but it is to some extent expensive. As a result farmers often become reluctant to ameliorate soil acidity. With this objective basic slag, a low cost liming material was undertaken to judge its suitability as an ameliorant of acid soil comparing calcite.

MATERIALS AND METHODS

Fourteen field experiments were conducted on farmers' field for wheat crop using K-9107 as a test variety in two districts (Midnapur West and Purulia) of red and lateritic tract (Alfisols) of West Bengal in the rabi season of the years 2003-04 and 2004-05 for this purpose. Lime requirement (LR) values of the experimental soils were also determined following SMP method [6]. The LR values in Table 1 gave the amount of $CaCO_3$ needed to neutralise the soil acidity. Another lime source i.e., basic slag was also used as a low cost locally available liming material. The equivalent amount of basic slag needed was also determined by calculating the relative neutralising power of basic slag vis-a-vis calcite. An average composition of basic slag was: Ca (33.2%), Mg (3.2%), P_2O_5 (2.1%), Si

(2.3%), Cu ($163 \mu g g^{-1}$) and Zn ($174 \mu g g^{-1}$). Three doses viz., no lime, lime @ $1/5^{th}$ LR (lime requirement value) and lime @ $1/10^{th}$ LR of both the liming materials were used for the experiment. Besides liming materials, locally available organic sources e.g. farmyard manure (FYM) and poultry manure (PM) were incorporated with basic slag in all the places with the assumption that they might help to increase the solubility of basic slag. Thus the treatments used were as follows: No lime, $1/5^{th}$ LR (basic slag), $1/10^{th}$ LR (basic slag), $1/5^{th}$ LR (calcite), $1/10^{th}$ LR (calcite), $1/5^{th}$ LR (basic slag + FYM @ 5t/ha) and $1/5^{th}$ LR (basic slag + PM @ 3t/ha). Crop was sown with the recommended doses of N, P and K (@ 80:40:40 kg ha $^{-1}$). Full dose of P and K were applied at the time of sowing but for N half of the recommended dose was applied at the time of sowing and the rest half of N was applied at 21 DAS i.e. at crown root initiation stage. Calculated amount of the liming material corresponding to the three doses was mixed up with the soils in the furrows. After harvest grain and straw yield of the crop were recorded and the plant samples were analysed for N, P and K following standard methods. Economy of the liming materials used was also calculated.

Soil samples both the initial and residual (collected after harvest of the crop) were analysed for soil pH (both pH_w and pH_{ca}) and different forms of soil acidity viz., total acidity, exchange acidity, hydrolytic acidity, electrostatically bound H^+ (EBH^+) and Al^{3+} ($EBAI^{3+}$). Total acidity (TA) and exchange acidity (EA) were determined by extracting soil with 1.0 M sodium acetate (pH 8.2) [7] and 1.0 M KCl [8] respectively and subsequently titrating with NaOH using phenolphthalein as an indicator. Electrostatically bound Al ($EBAI^{3+}$) was determined in 1.0 M KCl extract by titrating with HCl after adding NaF. The difference between EA and $EBAI^{3+}$ represented the electrostatically bound H^+ (EBH^+). The difference between total acidity (TA) and exchange acidity (EA) was designated as hydrolytic acidity (HA) [9].

RESULTS AND DISCUSSION

All the soils used in the experiment were acidic in nature with mean pH values of 5.1 (pH_w) and 4.4 (pH_{ca}) (Table 1). Lower values of pH_{ca} than pH_w explained that the soils were negatively charged. The total acidity (TA) of the soils as extracted by 1.0 M NaOAc, pH 8.2 varied from 1.31 - 2.57 cmol (p^+) kg $^{-1}$ with mean values of 1.76 cmol (p^+) kg $^{-1}$ (Table 1). The hydrolytic acidity (HA) varied from 1.0 to 1.95 cmol (p^+) kg $^{-1}$ with a mean value of 1.42 cmol (p^+) kg $^{-1}$. The exchange acidity (EA) includes

Table 1: Forms of acidity and lime requirement values of the experimental soils before lime application

	Total acidity	Exchange acidity	Hydrolytic acidity	EBAl ³⁺	EBH ⁺	pH _w	pH _{ca}	LR (t haG ¹)
Experimental sites	[cmol (p ⁺) kgG ¹]							
Pukuria	1.43	0.32	1.11	0.21	0.11	5.3	4.5	4.70
Radhanagar	1.31	0.14	1.18	0.05	0.09	5.2	4.6	6.17
Dhenkipora	2.57	0.97	1.60	0.54	0.42	4.8	4.3	4.70
Dahijuri	2.19	0.86	1.33	0.51	0.34	5.1	4.1	7.66
Andharia	2.37	0.90	1.47	0.55	0.34	5.1	4.2	7.66
Binpur	1.33	0.23	1.10	0.15	0.09	4.9	4.5	4.70
Kapgari	1.39	0.14	1.25	0.12	0.02	5.3	4.7	6.17
Bansra	1.33	0.33	1.00	0.30	0.03	5.2	4.1	6.17
Gopladih	1.75	0.10	1.65	0.04	0.06	5.1	4.7	3.45
Manikdih	1.52	0.19	1.33	0.06	0.13	5.3	4.3	7.66
Sirkabad	1.73	0.10	1.64	0.03	0.07	4.7	4.5	7.66
Santladih	2.24	0.29	1.95	0.22	0.07	5.2	4.2	6.17
Govindpur	1.88	0.08	1.80	0.03	0.06	5.0	4.6	4.70
Chakaltod	1.60	0.07	1.53	0.01	0.06	5.0	4.4	4.70
Range	1.31-2.57	0.07-0.97	1.0- 1.95	0.03-0.55	0.02-0.42	4.7-5.3	4.1-4.7	3.45-7.66
Mean	1.76	0.34	1.42	0.20	0.14	5.1	4.4	5.88
SD	0.42	0.32	0.28	0.20	0.13	0.19	0.20	1.41

LR= Lime requirement of soil in the form of CaCO₃

Table 2: Effect of liming on grain yield of wheat (q haG¹)

Experimental sites	No lime	BS 1/5 th	BS 1/10 th	Ca 1/5 th	Ca 1/10 th	BS 1/5 th + PM	BS 1/5 th + FYM
Pukuria	13.7	23.5	19.1	21.5	15.8	25.1	24.5
Radhanagar	19.3	26.8	21.5	24.5	20.2	28.5	28.0
Dhenkipora	18.2	25.5	20.5	23.3	19.2	28.1	27.4
Dahijuri	16.0	27.5	18.5	25.8	18.2	28.9	28.1
Andharia	18.5	22.6	20.5	21.5	19.3	26.7	25.5
Binpur	17.3	24.2	19.8	20.8	18.5	27.3	26.1
Kapgari	18.5	23.5	20.6	22.8	19.5	27.5	26.4
Bansra	17.8	22.4	20.7	23.7	19.6	25.8	24.3
Gopladih	14.4	25.3	16.2	23.0	18.1	28.6	27.4
Manikdih	16.5	24.5	20.0	21.9	18.8	26.8	25.3
Sirkabad	18.0	23.8	20.2	23.7	19.5	26.3	25.1
Santladih	16.0	27.3	22.5	25.0	17.0	29.5	28.2
Govindpur	17.4	26.2	20.2	24.5	19.3	28.1	27.5
Chakaltod	17.3	24.7	20.0	22.2	18.8	26.7	25.3
Mean	17.1	24.8	20.0	23.2	18.7	27.4	26.8
Se _m (±)	0.395						
CD (P= 0.05)	1.116						

the exchangeable H⁺ and Al³⁺ held at the permanent charge sites of the soil exchange complex. Unlike TA, the EA of all the soils was much less and its value ranged from 0.07 to 0.97 cmol (p⁺) kgG¹ with a mean value of 0.34 cmol (p⁺) kgG¹. Soils were limed on the basis of lime requirement (LR) estimated.

Effect of lime on grain and straw yield: Results showed that application of lime caused a significant increase in grain (GY) and straw yield (SY) of wheat (Table 2 & 3). The magnitude of increase in GY and SY due to liming was 26.8 and 18.6 per cent respectively over the no lime treatment, irrespective of the sources and levels of lime.

Table 3: Effect of liming on straw yield of wheat (q ha⁻¹)

Experimental sites	No lime	BS 1/5 th	BS 1/10 th	Ca 1/5 th	Ca 1/10 th	BS 1/5 th + PM	BS 1/5 th +FYM
Pukuria	21.8	29.3	25.0	26.7	23.3	32.4	31.5
Radhanagar	25.5	31.2	27.2	30.2	25.6	33.1	32.6
Dhenkipora	23.3	30.9	25.5	28.5	23.8	33.8	33.4
Dahijuri	24.8	33.8	30.5	30.5	28.3	36.3	35.4
Andharia	25.4	31.2	29.1	30.6	27.2	34.2	33.6
Binpur	21.5	26.8	24.6	26.4	23.0	29.1	28.5
Kapgari	22.4	29.4	27.1	27.6	25.3	31.6	30.9
Bansra	25.5	34.2	26.3	31.2	24.2	36.8	36.1
Gopladih	20.5	29.5	22.7	33.2	21.1	32.2	31.5
Manikdih	23.3	31.5	27.5	27.2	25.5	33.5	32.6
Sirkabad	24.3	28.8	31.2	30.2	28.6	31.0	30.2
Santladih	25.6	33.2	30.2	29.5	27.5	34.8	34.1
Govindpur	26.1	31.6	26.5	29.2	24.1	34.2	33.5
Chakaltod	21.6	28.5	25.5	25.6	23.3	31.2	30.2
Mean	23.7	30.7	27.1	29.0	25.1	34.1	33.5
Se _m (±)	0.579						
CD (P= 0.05)	1.635						

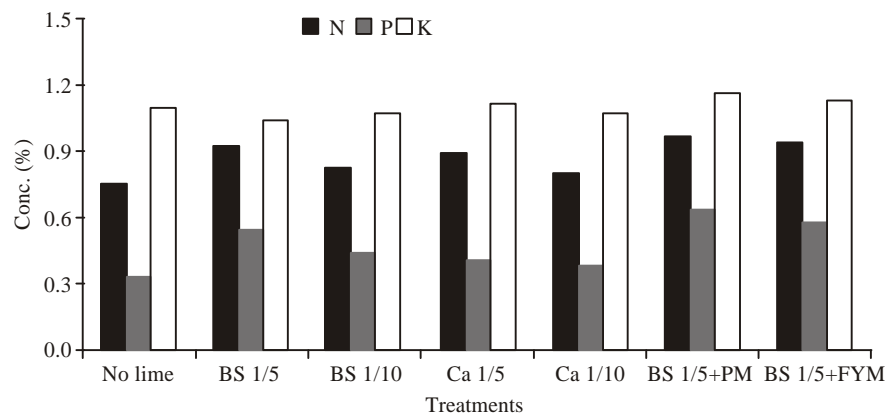


Fig. 1: N, P and K content in wheat plants (mean of 14 experiments)

Such increase in both GY and SY was always higher with basic slag than with calcite, the magnitude of increase in GY and SY being 31.0 and 21.9 per cent with the former but 22.5 and 14.1 per cent with the latter (Table 2 & 3). Results thus indicated a better response of wheat to basic slag than calcite. Response of wheat to liming also varied depending upon their levels of application. There was a higher response with higher doses of lime, the mean magnitude of GY and SY being 17.1, 19.4, 24.0 and 23.7, 26.1, 29.9 q ha⁻¹ with no lime, LR1/10th and LR 1/5th levels of added lime respectively (Table 2 & 3). These constituted an increase in GY of about 13.5 and 40.6 per cent over the control with LR1/10th and LR1/5th levels of lime respectively. The corresponding values for SY were 10.0 and 26.1 per cent. The magnitude of increase with basic slag @ 1/5th LR was further enhanced when it was incorporated either with FYM or PM. Incorporation of FYM and PM caused a yield increase of 56.7 and 60.2% respectively over the no lime and 19.6 and 22.3% over the

only basic slag @ 1/5th LR treatment. Straw yield also showed the similar trend of results. Significant increase in grain yield of maize on liming even with 1/4th lime requirement value were recorded [10]. Increase in yield with higher doses of liming material was observed by [11, 12, 13]. The relative order of performance of the treatments was as follow: 1/5th LR (basic slag + PM @ 3t/ha) > 1/5th LR (basic slag + FYM @ 5t/ha) > 1/5th LR (basic slag) > 1/5th LR (calcite) > 1/10th LR (basic slag) > 1/10th LR (calcite) > and no lime. Results thus showed that locally available organic resources like FYM and PM would be effective in increasing the efficacy of basic slag for increasing the productivity of wheat crop in acidic Alfisols of West Bengal.

N, P and K content in wheat plants: Results (Fig. 1) showed that liming caused significant increase in N and P content of crop. Application of lime caused a significant increase in N concentration in wheat plants.

Table 4: Changes in soil acidity parameters [cmol (p⁺) kgG¹] after harvest of wheat crop

Treatments	pHw	pHca	EB Al ³⁺	EB H ⁺	Total acidity	Exchange acidity	Hydrolytic acidity
Initial	5.1	4.4	0.20	0.14	1.76	0.34	1.42
No lime	5.2	4.5	0.18	0.12	1.66	0.31	1.35
BS 1/5 th LR	6.0	5.6	0.04	0.05	0.84	0.09	0.75
BS 1/10 th LR	5.5	5.0	0.09	0.07	1.14	0.16	0.98
Ca 1/5 th LR	6.1	5.9	0.03	0.04	0.56	0.07	0.49
Ca 1/10 th LR	5.7	5.2	0.07	0.07	1.05	0.14	0.92
BS 1/5 th LR + PM	6.3	5.9	0.08	0.07	0.90	0.15	0.81
BS 1/5 th LR + FYM	6.2	5.8	0.07	0.06	0.89	0.13	0.76
Mean	5.8	5.3	0.10	0.08	1.10	0.17	0.94
Se _m (±)	0.039	0.031	0.025	0.019	0.081	0.042	0.075
CD (P= 0.05)	0.110	0.110	0.071	0.054	0.229	0.119	0.212

(Mean of fourteen experiments)

Table 5: Economics of lime application in wheat crop

Treatment	Price of lime (Rs)	Yield (q haG ¹) *	Yield increased over check (q)	Percent response	Price of increased yield (Rs)	Profit over check (Rs haG ¹)	Return in per Re investment (B: C)
No lime	-	17.1	-	-	-	-	-
BS 1/5 th LR	1093	24.8	7.7	45.6	6223	5130	4.7
BS 1/10 th LR	546	20.0	2.9	17.6	2366	1820	3.3
Ca 1/5 th LR	2352	23.2	6.1	35.7	4874	2522	1.1
Ca 1/10 th LR	1176	18.7	1.6	9.4	1309	133	0.1
BS 1/5 th LR + PM	1093	27.4	10.3	60.2	8240	7147	6.5
BS 1/5 th LR + FYM	1093	26.8	9.7	56.7	7760	6667	6.1

(mean of 14 experiments, price of wheat @ Rs 8/- per kg, price of basic slag Rs 80/- per quintal, price of calcite @ Rs 200/- per quintal)

The mean increase of N concentration was 17.1 per cent over the no lime. Such increase was higher with basic slag than with calcite; the magnitude being 19.7 per cent and 11.8 per cent over the no lime respectively (Fig 1). Concentration of N in wheat plants also varied depending upon the levels of lime application. The concentration was higher with higher dose LR 1/5th, the magnitude of increase being 22.4 and 9.2 per cent with LR 1/5th and LR 1/10th doses respectively. There was a significant increase in the concentration of N when the organic residues like FYM and PM were incorporated with basic slag. The magnitude of increase was 23.7 and 27.6 per cent with FYM and PM respectively. Highest concentration of N (0.97%) was observed in the treatment LR 1/5th basic slag + PM. This indicates a better nutrition of N nutrition of wheat plants when acid soils are limed. Increase in availability and plant uptake N was also reported by Curtin and Smillie [14], Barade and Chavan [15] and Raychadhury *et al.* [16]. Application of lime also caused significant increase in P concentration (Fig. 1) in wheat plants, the mean increase being 51.5 per cent over the no lime. Such increase in P concentration on liming was higher with basic slag than with calcite, the magnitude being 65.9 and 19.7 per cent respectively. The application of FYM and PM with basic slag caused a significant

increase in the concentration of P in plants. The results, therefore, indicated that a better response of wheat crop in respect of P nutrition was observed in limed soils than in the unlimed soils. Increased P availability and uptake by different crop plants upon liming was reported by Patiram Rai and Prasad [17], and Mongia *et al.* [18]. Application of lime did not show any specific trend of increase or decrease in concentration of K (Fig. 1) in wheat plants. The concentration of K was increased to about 0.92 per cent in limed soils. Such increase in concentration was higher when calcite and basic slag were used at lower doses. Significant increase in K content of wheat was observed with the application of organic manures. Results thus indicated that liming showed a mixed responses in K concentration by wheat plants. Decrease in K availability on liming was also observed by Prasad *et al.* [19] and Dwivedi [20].

Analysis of residual soils: Soil samples collected after the harvest of wheat crop were analysed for different soil properties viz., pH_w, pH_{ca}, OC and a few acid parameters such as total acidity, exchange acidity and hydrolytic acidity were also analysed to estimate the changes that occurred upon liming. Results (Table 4) showed that application of amendments caused

significant increase in both pH_w and pH_{ca} . The mean magnitude of increase was 0.8 and 1.0 unit for pH_w and pH_{ca} irrespective of doses and sources of lime respectively. The increase was higher (0.9 unit) when calcite 1/5th LR was used as compared to the magnitude of increase (0.7 unit) with basic slag 1/5th LR. There are a number of reports that addition of organic residues to acid soil can reduce Al toxicity (thus lowering the lime requirement) and improve P availability. Significant increase in soil pH with the application of organic sources was observed. During residue decomposition, there is a transitory increase in soil pH and this induces a decrease in exchangeable and soil solution aluminium through their precipitation as hydroxy-Al compounds. It also confers a great negative charge on oxide surfaces and thus tends to decrease P adsorption. Result of acid parameters (Table 4) showed that there was a significant decrease in total acidity, exchange acidity and hydrolytic acidity upon liming. Marked decrease of exchangeable Al upon liming at the rate of 25% of LR was observed by Prasad *et al.* [10]. Increase in pH upon liming was also reported by Datta and Gupta [21], Dhadwal *et al.* [22] and Prasad *et al.* [19]. Results thus indicate that basic slag @1/5th LR caused significant decrease in most of acid parameters in soils.

Economics of lime application: The economics was calculated only for the lime application, because the motive of the research was only to see the effect of liming materials with different doses over the No-lime treatment. Results (Table 5) showed that there was a net benefit out of application of lime. The benefit was more with basic slag than with calcite. With the application of basic slag @ 1/5th LR the B:C ratio was 4.7 as compared to 3.3 with lower dose 1/10th LR. Benefit cost ratio with calcite was 1.1 and 0.1 with @ 1/5th LR and 1/10th LR respectively. There was further increase in grain yield when FYM and PM were applied in combination of basic slag. Value cost and benefit cost ratio was fairly higher when basic slag was applied with PM followed by FYM. Results thus showed that use of basic slag as a liming source was more economical as compared to calcite in Alfisols of West Bengal. It has been mentioned earlier that basic slag contains, in addition to Ca and Mg, good amount of P, Si, Zn and Cu. Since most of the Alfisols of West Bengal are deficient to marginal in respect of P, Zn and Si, such application of P, Zn and Si along with Ca and Mg through basic slag helped to have a better response and economics. This was more so because of low cost of basic slag. Liming in Alfisols

through basic slag will thus be a good avenue for increasing productivity of these other-wisely low productive soils.

CONCLUSIONS

From the results it is revealed that use of basic slag as a source of lime will be very much effective to increase as well as to sustain the productivity of acid red and lateritic soils of West Bengal. Results also revealed that B:C ratio of basic slag is higher as compared to calcite. So it will be highly acceptable and affordable to the farmers of the area.

ACKNOWLEDGEMENT

The financial support provided by the Indian Council of Agricultural research (ICAR) through the Network Project on "Soil characterisation and resource management of acid soil regions for increasing productivity" is duly acknowledged.

REFERENCES

1. Shainberg, I., M.E. Sumner, W.P. Miller, M.P.W. Farina, M.A. Pavan and M.V. Fey, 1989. Use of gypsum on soils: a review. *Advances in Soil Science*. 9: 1-11.
2. Von Uexkull, H.R. and E. Mutert, 1995. Global extent, development and economic impact of acid soils. *Plant Soil*, 171: 1-15.
3. Sharma, P.D. and A.K. Sarkar, 2005. Managing acid soils for enhancing productivity. NRM Division, ICAR, New Delhi. Technical Bulletin, pp: 23.
4. Bandyopadhyay, P.K. and G.N. Chattopadhyay, 1997. Nature of acidity in some alfisols and inceptisols of Birbhum district of West Bengal. *Journal of the Indian Society of Soil Science*, 45: 5-8.
5. Somani, L.L., K.L. Totawat and R.A. Sharma, 1996. Liming Technology for Acid Soils. Agrotech. Publishing Academy, Udaipur, pp: 240.
6. Shoemaker, H.E., E.O. McLean and P.F. Pratt, 1961. Buffer methods for determining lime requirement of soil with appreciable amounts of extractable aluminium. *Soil Science Society of America Proceedings*, 25: 274-277.
7. Kappen, G., 1934. *Pochvennaya, Kislotmost, Selkhogi*, Moscow.

8. McLean, E.O., 1965. Aluminum. In C.A. Black (ed.) *Methods of Soil Analysis*, Part II: Chemical and microbiological properties of soils. Am. Soc. of Agron., Inc. Madison, Wis. pp: 978-998.
9. Peech, M., R.L. Cowan and J.H. Baker, 1962. A critical study of the BaCl₂-Triethanolamine and ammonium acetate methods for determining the exchangeable hydrogen content of soil. *Soil Society of America Proceedings*, 26: 37-40.
10. Prasad, R.N., Patiram Rai, R.C. Barooah and Munna Ram, 1983. Direct and residual effect of liming on yield of maize and uptake of nutrients in acid soils of Meghalaya. *Journal of the Indian Society of Soil Science*, 31: 233-235.
11. Bezdicsek, D.F., T. Beaver and D. Granatstein 2003. Subsoil ridge tillage and lime effects on soil microbial activity, soil pH, erosion and wheat and pea yield in the Pacific Northwest, USA. *Soil Tillage Research*, 74: 55-63.
12. Tang, C., Z. Rengel, E. Diatloff and C. Gazey, 2003. Responses of wheat and barley to liming on a sandy soil with subsoil acidity. *Field Crop Research*, 80: 235-244.
13. Bhat, J.A., A.K. Chatterjee and B. Mandal, 2004. Performance of different liming materials in increasing yield of mustard and ameliorating acidity in red and laterite soils of West Bengal. In: National Seminar on Developments in Soil Science, 69th Annual Convention Indian Society Soil Science held during Oct 29 - 31, 2004 at ANGRAU, Hyderabad, AP.
14. Curtin, D. and G.W. Smillie, 1995. Effects of incubation and pH on soil solution and exchangeable cation ration. *Soil Science Society of America Journal*, 59: 1006-1011.
15. Barade, M.D. and K.N. Chavan, 1998. Liming induced changes in characteristics of fluventic ustrochrept of Konkan. *Journal of the Indian Society of Soil Science*, 46: 5-8.
16. Raychadhury, M., Kailashkumar and S. Raychaudhury, 1998. Response of soybean to lime and phosphorus on Udisols of manipu. *Journal of the Indian Society of Soil Science*, 46: 628-632.
17. Patiram Rai, R.N. and R.N. Prasad, 1990. Effect of liming on aluminium and yielded of wheat in acidic soil. *Journal of the Indian Society of Soil Science*, 38: 719-722.
18. Mongia, A.D., N.T. Singh, L.N. Mandal and A. Guha, 1998a. Response of rice to liming and phosphorus application in acid soils of south Andaman. *Journal of the Indian Society of Soil Science*, 46: 697-700.
19. Prasad, R.N., R.N. Patiram, Rai and Munna, Ram, 1985. Forms of aluminium in soils of East Khasi Hills, Meghalaya. *Journal of the Indian Society of Soil Science*, 33: 523-527.
20. Dwivedi, G.K., 1996. Tolerance of some crops to soil acidity and response to liming. *Journal of the Indian Society of Soil Science*, 44: 736-741.
21. Datta, M. and R.K. Gupta, 1983. Response of wheat and maize to lime in acid soils of Nagaland. *Journal of the Indian Society of Soil Science*, 31: 236-240.
22. Dhadwal, K.S., S. Laskar and R.N. Prasad, 1985. Factros affecting lime requirement of acid soils of Tripura and response of soybean to liming. *Journal of the Indian Society of Soil Science*, 33: 683-684.

Biometry and Responses of Faba Bean Varieties to Black Bean Aphid, *Aphis fabae* Scopoli

Hail K. Shannag and Ja'far A. Ababneh

Department of Plant Production, Faculty of Agriculture,
Jordan University of Science and Technology, Irbid 22110, Jordan

Abstract: Influence of the black bean aphid, *Aphis fabae* Scopoli, on the growth of different faba bean varieties, namely; 79S4, S82408-1-2-3, Aquadulce, FLIP87-26FB, *Vicia faba major* and *Vicia faba minor* was investigated under semiarid field conditions. Three late-nymphal instars of aphid were used to infest individual plant at 28 days after plant emergence. Results showed that aphid-infested plants were reduced in all growth parameters tested and the magnitude of damage relied on the variety. An enormous decrease in the shoot fresh and dry weights, leaf area and plant height were recorded for *V. faba major* and Aquadulce, while *V. faba minor* variety tolerated the aphid attack. The number of aphids increased exponentially at an early growth stage of *V. faba major* and Aquadulce, causing ultimately plants to die and thus aphid populations crashed. On other varieties, aphids propagated incessantly, reaching a peak at days 56 days after artificial infestation, but the infestation rates were variable with reliance on variety. Subsequently aphid populations declined steadily until the end of the growing season. Aphid-free varieties fluctuated in their growth rates during the study. S82408-1-2-3, 79S4 and FLIP87-26FB varieties produced overall plants with maximum sum of plant height, shoot fresh and dry weights, as well as leaf area, whereas *V. faba minor* was at least.

Key words: *Aphis fabae* % faba bean % plant growth

INTRODUCTION

Faba bean, *Vicia faba* L., is one of the most important legume crops around the globe [1]. In the Mediterranean region, faba bean is a staple food and cheap source of high quality protein for most population [2]. It is considered also as a great prolific animal resource as feed to all types of livestock [3] and used to make a silage of high quality in some countries [4]. Faba bean is capable to fix atmospheric nitrogen through the symbiotic relationship with *Rhizobium*-bacteria and so improves the nitrogen status in soil [5].

In Jordan, faba bean is the most common and widely used legume after lentil. The area planted to this crop under both rainfed and irrigation conditions comprises approximately 14% of the total area seeded to legumes [6]. However, the total production of faba bean is still low and far below the country's needs. In spite of the increasing demand for the faba bean in the country, the area designated to this crop and the annual production are decreasing due to low and erratic rainfalls, planting traditional low yielding cultivars, poor cultural practices

and pest infestation [7]. Moreover, the black bean aphid, *Aphis fabae* Scopoli, is a major constraint of faba bean production, which inflicts a destructive damage to faba bean throughout the world. In addition to direct plant injury, aphid infestation harms extensively faba bean by honeydew excretion, which stimulates the growth of sooty mold. Honeydew deposited on the leaves interferes with some physiological processes in the host plant [8].

The high damage potential and unpredictability of *A. fabae* infestation usually lead to an extensive pesticide application based often on a fixed schedule. However, there are significant economical, environmental and health cost associated with this approach, which result in an increasing awareness of usefulness of integrated pest management schemes in which host plant resistance must have a central role. Several authors have recognized the potential value of plant resistance for controlling *A. fabae* and therefore some partially resistant faba bean cultivars were identified against this aphids [9-13]. However, high levels of resistance were detected only in landraces, progenies and wild relatives of *V. faba* [14].

The present study was conducted to assess the responses of different faba bean varieties to the infestation by *A. fabae* under semiarid field condition, as indicated by the measurements of shoot fresh and dry weight, leaf area and plant height.

MATERIALS AND METHODS

Stock of black bean aphid, *A. fabae*, was collected from infested fields of faba beans in the Jordan Valley, Jordan. Aphids were reared on potted faba bean plant, *V. faba major* under organdy screened cages (80×60×60 cm), in an insectary at a temperature of 20±3°C, 46-80% relative humidity and 16L:8D photoperiod. New faba bean plants grown under greenhouse conditions were added when old plants senesce as a result of high feeding pressure of aphids. In order to infest the experimental plants with similar aged aphids, a synchronized colony of *A. faba* was established. Apterous adults were transferred from stock colony onto two-week old *V. faba* plants placed in a new cage. Cages were covered in sides with organdy screen and the top with transparent plastic sheet. Aphids retained on the plants for 4-5 h to produce progeny. Then, adult aphids were removed and the offspring were allowed to develop until they reach late-nymphal instars (8-days).

Seeds of faba bean varieties; 79S4, S82408-1-2-3, Aquadulce and FLIP 87-26-FB, provided by International Center for Agricultural Research in Dry Area (ICARDA) and two wide cultivated varieties in the region, *V. faba major* and *V. faba minor*, were grown in the field on Jordan University of Science and Technology campus, Irbid, Jordan. Seeds were hand planted in three rows per plot with 30 inter row space and 20 cm intra row space. Plants were watered by a drip irrigation system and fertilized by diammonium phosphate (18N-46P-0K) at rate of 30 kg ha⁻¹ prior to seeding. Weeds were removed manually as needed.

Split plot design with four replicates was used in this experiment. Each block was divided into main plots with six units (subplots) of 1 m² with protection spacing of one meter between the units. Faba bean varieties were distributed randomly in each unit, each one contained 12 plants. At the time of aphid infestation, plots were randomly arranged into two groups, control and infested. Each experimental plant in infested group was infested at 28 days after plant emergence by three fourth nymphal instars (8 days old) obtained from a synchronized colony. Control plants remained aphid-free. Immediately after aphid release, all treatments including control were covered with organdy-screen cages, each measuring 1L×1W×1H m.

Three plants from each replicate were randomly sampled at 21, 42, 63 and 84 days after the artificial infestation. Sampled plants were cut direct above ground, placed individually in plastic bags and, thereafter, the plant height and shoot fresh weight were measured in the laboratory. Plants were then dried separately in drying oven at 68°C for 48 hrs and shoot dry weight was weighed. Leaf area of each plant was determined using a leaf area meter type LI-3000 area meter (Li-Cor. Inc., Lincoln, NE). Number of aphids was estimated at two-week intervals during the study. Data were subjected to analyses of variance (Two way ANOVA) using MSTATC software (Michigan State University, 1988). Means were compared using Fisher's least significant differences (LSD) test at a 0.05 probability level.

RESULTS

Aphid populations on different faba bean varieties: *A. fabae* populations on different faba bean varieties are illustrated in Table 1. Results indicated that there were differences in the development of aphid populations between faba bean varieties. Aphids started to increase

Table 1: Population growth of *Aphis fabae* on six faba bean varieties under semiarid field conditions

Varieties	Number of aphids after					
	14 days	28 days	42 days	56 days	70 days	84 days
79S4	163.3a	450.0a	2417.0ac	5267.0a	3067.0a	701.0a
S82408-1-2-3	155.0b	445.0a	2217.0a	6000.0b	3867.0b	504.0b
Aquadulce	193.3c	983.3b	5400.0b	9800.0c	-	-
FLIP87-26FB	180.0b	600.0c	2983.0c	5350.0a	3400.0c	633.3a
Vicia faba major	205.0c	1200.0d	7917.0d	-	-	-
Vicia faba minor	175.0b	1033.0b	4283.0e	6650.0d	4750.0d	833.3c
LSD	5.99	62.84	597.9	266.5	192.4	68.74

Means followed by same letter(s) within each are not significantly different at p = 0.05

Table 2: Average plant height (cm) of different faba bean varieties infested by *Aphis fabae* for different periods of time

Varieties	Days after infestation											
	21			42			63			84		
	Control	Infested	Red. (%)	Control	Infested	Red. (%)	Control	Infested	Red. (%)	Control	Infested	Red. (%)
79S4	54.0a	40.5*	25.0	63.7a	60.9	4.4	76.5a	68.9*	9.9	82.5a	69.9*	15.2
S82408-1-2-3	54.7a	44.5*	18.7	61.5a	52.1*	15.3	75.9a	68.6*	9.6	78.6a	70.0*	10.9
Aquadulce	60.2a	39.2*	34.9	64.2a	46.2*	28.0	78.1a	48.7*	37.6	-	-	-
FLIP87-26FB	56.0a	47.8*	14.7	59.7a	49.3*	17.4	68.8b	60.2*	12.5	73.1b	62.1*	15.0
<i>Vicia faba major</i>	56.7a	41.7*	26.5	58.8a	49.0*	16.7	-	-	-	-	-	-
<i>Vicia faba minor</i>	56.0a	47.3*	15.5	60.5a	49.0*	19.0	63.6b	54.3*	14.6	67.7c	58.7*	13.3
LSD	7.381			7.094			5.312			5.045		

Means followed by same letter (s) within each date are not significantly different at $p = 0.05$, Numbers joined with (*) are significantly different from the respective control at $p = 0.05$

obviously in the number at 28 days followed aphid release, reaching a peak of day 56. *A. fabae* population was mainly abundant on *V. faba major* during the first six weeks and its number exceeded significantly the aphid populations on the other varieties, apart from Aquadulce on 14 and 28 days. Aphid quantity on Aquadulce ranked in the second place, increasing significantly at 28, 42 and 56 days with respect to other treatments, excluding *V. faba minor* over 28 days. The growth of these tremendous aphid populations at an early stage of *V. faba major* and Aquadulce development caused these both varieties to die prematurely and the aphid populations on them to collapse at 42 and 56 days respectively. However, aphids achieved a maximum number on *V. faba minor*, FLIP87-26FB, 79S4 and S82408-1-2-3 varieties at 56 days which later dropped steadily until the end of growing season. Among these still alive varieties, aphids developed significantly a greater number on *V. faba minor* than the individuals on 79S4, S82408-1-2-3 and FLIP87-26FB during all monitoring dates, apart from days 14. On day 42, there were no significant differences in aphid numeral between 79S4 and S82408-1-2-3 varieties. However, aphid densities on S82408-1-2-3 variety exceeded significantly those on FLIP87-26FB and 79S4 at days 56 and 70, but decreased to a minimum on day 84.

Biometry of aphid-infested *V. faba* varieties

Effect of *A. fabae* on plant height: Results indicated that aphid-free varieties showed clear differences in the plant height during the growing season (Table 2). After 42 days, aphid-free faba bean varieties did not differ significantly among each other. However, FLIP87-26FB, *V. faba minor* and *V. faba major* varieties were more reduced in the plant height than other varieties on day 63. At the

end of growing season (84 days), all tested varieties fluctuated significantly in the plant height among each other where 79S4 variety produced the tallest plants and *V. faba minor* was the shortest one.

Aphid attack harmed considerably the plant height on all sampling dates with respect to the relevant controls, except for 79S4 variety at 42 days (Table 2). This reduction ranged between 4-38% depending on variety and infestation interval. Aquadulce variety was most impaired by aphid feeding, showing a 28-38% decrease in the plant height in comparison with respective control.

Responses of shoot fresh and dry weights to aphid infestation: Aphid-free faba bean varieties varied remarkably in the shoot fresh weight among each other during the experiment (Table 3). After 21 days, 79S4, Aquadulce and FLIP87-26FB varieties produced the greatest shoot fresh weight, while *V. faba minor* was as a minimum. Three weeks later (42 days), however, the shoot fresh weights were about equal by all varieties, except for minor weight of *V. faba minor* variety. By day 63, the average shoot fresh weight of 79S4 and S82408-1-2-3 was greater than those of other varieties. However, at the last sampling date still alive faba bean varieties did not show significant differences among each other.

In all treatments, aphid infestation induced a 9-61% decline in the shoot fresh weight with reliance on variety and infestation period. Aphid feeding induced significant reductions in this parameter on faba bean varieties at 21 and 63 days. *V. faba major* variety suffered actually from aphid attack more than other varieties showing evidence of 62% and 44% decline in the fresh weight on 21 and 42 days, respectively (Table 3). In general, injury levels were

Table 3: Effect of *Aphis fabae* on the shot fresh weight of different faba bean varieties at different infestation times

Varieties	Days after infestation											
	21			42			63			84		
	Control	Infested	Red. (%)	Control	Infested	Red. (%)	Control	Infested	Red. (%)	Control	Infested	Red. (%)
79S4	194.9a	68.2*	65.0	210.2a	192.0	8.7	222.3a	170.1*	23.5	243.8a	191.8*	21.3
S82408-1-2-3	151.2bc	64.9*	57.1	217.1a	193.3	11.0	241.3a	180.4*	25.3	249.5a	177.4*	28.9
Aquadulce	192.5a	65.6*	65.9	202.8ab	154.6*	23.8	207.4ab	145.7*	29.7	-	-	-
FLIP87-26FB	187.0ab	136.1*	27.2	208.1ab	184.4	11.4	219.0a	140.1*	36.0	232.3a	203.9	12.2
<i>Vicia faba major</i>	153.6b	59.0*	61.6	176.6bc	98.5*	44.2	-	-	-	-	-	-
<i>Vicia faba minor</i>	116.7c	55.7*	47.7	156.2c	112.5*	28.0	176.1b	103.7*	41.1	212.7a	193.5	9.0
LSD	36.06			32.51			36.71			44.07		

Means followed by same letter(s) within each date are not significantly different at p = 0.05, Numbers joined with (*) are significantly different from the respective control at p = 0.05

Table 4: Average shoot dry weight (g) of aphid-free and *A. fabae*-infested faba bean varieties at different times after aphid infestation

Varieties	Days after infestation											
	21			42			63			84		
	Control	Infested	Red.%	Control	Infested	Red.%	Control	Infested	Red.%	Control	Infested	Red.%
79S4	26.42a	8.72*	67.0	32.07ab	27.87*	13.1	33.80a	28.67*	15.2	35.40a	31.03*	12.3
S82408-1-2-3	22.99b	9.60*	58.2	33.82a	27.87*	17.6	34.80a	29.67*	14.7	36.37b	31.77*	12.6
Aquadulce	26.32a	9.14*	65.3	29.87b	24.33*	18.5	30.87b	26.67*	13.6	-	-	-
FLIP87-26FB	21.97b	11.44*	47.9	30.95ab	24.87*	19.6	31.60b	26.53*	16.0	32.60c	28.93*	11.3
<i>Vicia faba major</i>	21.34b	6.26*	70.7	29.60b	14.20*	52.0	-	-	-	-	-	-
<i>Vicia faba minor</i>	18.95c	6.27*	66.9	26.42c	17.97*	32.0	27.97c	20.00*	28.5	30.23d	25.38*	16.0
LSD	1.911	3.158	1.300	0.619								

Means followed by same letter(s) within each date are not significantly different at p = 0.05, Numbers joined with (*) are significantly different from the respective control at p = 0.05

more prominent on 21 days after aphid release, which ranged from 27.2 to 65.9% depending on variety.

Variations in the shoot dry weight were also apparent amongst aphid-free varieties during the whole plant growth period (Table 4). Shoot dry weight of *V. faba minor* was significantly lesser than other varieties throughout the experimental period, while S82408-1-2-3 and 79S4 varieties produced generally the highest dry weight. When aphids were confined to the plants, all varieties decreased obviously in the mean shoot dry weight (Table 4). However, damage level turn down commonly with the progressive plant growth. After 21 days, the relative dry weight of infested plants varied between 47.8-70.6% of the respective controls with reliance on varieties. 10-11.3% decrease in the dry weight was only recorded between still alive aphid-infested plants on 84 days. Sever damage was apparent on *V. faba major* prior to its death due to heavily aphid

infestation, followed by *V. faba minor* for the rest of experimental period. Other varieties, 79S4, S82408-1-2-3, Aquadulce and FLIP 87-26-FB, showed variable responses to aphid injury within all sampling dates.

Impact of aphids on leaf area: Aphid-free *V. faba* varieties demonstrated apparent differences in the leaf area among each other (Table 5). FLIP87-26FB variety generated significantly a greater leaf area than other varieties overall the experimental period, excluding at days 63. Minimum leaf area was produced by *V. faba major* in the first and the second sampling dates and then by *V. faba minor* for the rest of the growing season.

Also, aphid infestation impaired obviously the leaf area of fabae bean varieties. Significant differences in the leaf area were recorded between aphid-infested varieties and their respective controls in all sampling date, except for 79S4 on day 42 and FLIP87-26FB

Table 5: Mean leaf area (cm²) of different faba bean varieties infested with *A. fabae* after different infestation periods

Varieties	Days after infestation											
	21			42			63			84		
	Control	Infested	Red.%	Control	Infested	Red.%	Control	Infested	Red.%	Control	Infested	Red.%
79S4	981a	798*	18.7	1285a	1163	9.5	2246 a	1976*	12.0	2323a	2131*	8.3
S82408-1-2-3	1549b	1250*	19.3	1883b	1520*	19.3	2197 a	1969*	10.4	2288a	2129*	6.9
Aquadulce	1561b	1446	7.4	1733c	1082*	34.7	1866 b	1218*	34.7	-	-	-
FLIP87-26FB	2124c	1915*	9.8	2242d	2032*	9.4	2396 ac	2142*	10.6	2465b	2389	3.1
<i>Vicia faba major</i>	901 da	602*	33.2	990e	481*	51.4	-	-	-	-	-	-
<i>Vicia faba minor</i>	1072 ea	858*	20.0	1224a	975*	20.3	1622 d	1021*	37.1	2106c	1900*	9.8
LSD	119.37			131.53			106.98			78.62		

Means followed by same letter(s) within each date are not significantly different at $p = 0.05$, Numbers joined with (*) are significantly different from the respective control at $p = 0.05$

on day 84 (Table 5). In general, leaf area of *V. faba major* was harshly injured, with moderate damage on 79S4, S82408-1-2-3 and Aquadulce varieties. FLIP87-26FB variety was more tolerable to aphid attack than other varieties during the whole experimental period.

DISCUSSION

Aphid-free faba bean varieties fluctuated widely in the plant height, shoot fresh and dry weights, in addition to the leaf area under semiarid field conditions. S82408-1-2-3, 79S4 and FLIP87-26FB varieties showed in general the greatest vegetative growth rates, whereas *V. faba minor* was as a minimum. Substantial differences in the yield components were also recorded by Ishang [15] using other faba bean varieties and genotypes. These variations in the growth rates of faba bean varieties could be attributed to the different adaptation talents of crop variety for the environmental conditions prevailing during the experiments [16], as well as to the erratic genetic complements of varieties.

All the six tested varieties responded to heavy aphid infestation through reducing the plant biomass. With respect to the vegetative components examined thus far, *V. faba major* and Aquadulce varieties appear to response more sensitive to the reduction in shoot fresh and dry weights, leaf area and plant height, while *V. faba minor* is more tolerant to aphid attack. Changes in these growth parameters were more evident at days 21 after aphid infestation, which concurs, to a large extent, with finding of Prüter and Zebitz [13] using a combination of *A. fabae* with other faba bean varieties. In contrast, Hawkins *et al.* [17] ascertained the greatest reduction in *V. faba* growth rate on the first week as a result of infestation by

A. craccivora. These contrary results may indicate that the responses are specific to the plant-aphid combinations investigated

Sever damage to *V. faba major* and Aquadulce observed in the present study can be caused by the exponential increase in aphid populations at an early stage of plant development, which may exceeds the carrying capacity of aphid injury resulting ultimately in prematurely death of those both varieties. The other four varieties can be classified as the less attractive nourishment for *A. fabae*, since they delayed the development of aphid populations and therefore become more capable to overcome the sensitive growth stage at the beginning of infestation. In this case, the ratio of removed to produced assimilates during the further course of infestation is probably more advantageous for the growth of old plants [13].

Less favored plant varieties by aphids, sometimes referred as resistant or partially resistant varieties, have been reported to have deleterious effects on the reproductive rate, nymphal survival, longevity of original adults and development rate of aphids, including *A. fabae*, compared to the susceptible ones [9, 11, 18-20]. Changes in the host vulnerability to the black bean aphid have been partially referred to the chemical composition in the plant tissues, particularly the total free amino acids [9, 21-23] and/or morphological traits of the plant [24-26]. A low tolerance of *V. faba*, cv. Diana to *A. fabae* attack has been attributed to a prior high production potential of this cultivars, which does not allow any considerable increase to compensate for occurring injury, compared to resistant *V. faba*, cv. Bolero [13]. However, the resistance to different pests on one host might not be the same basis [18].

The mechanisms underlying the reduction of growth components of faba bean by aphid might include the removal of assimilates and adjusting the sink-source ratio to the benefit of aphids [27, 28]. The absolute decline of photosynthetic surface area of plants [29], the excretion, with aphid saliva, of toxic or phytohormone-analogue compounds [30, 31] and/or a combination of these factors [32] may be also accountable for the reduction in plant biomass. Besides these reasons, both honeydew deposited on the leaves and the growth of sooty molds can hamper photosynthesis, transpiration and respiration of host plant [33].

Moreover, aphid populations did not increase incessantly during the whole experiment, but a decrease in aphid numbers on less sensitive varieties started after 59 days. This reduction could be caused by altering host plant to an inferior food source for aphids under heavy infestation [34] and/or by obligating aphid individuals to compete with each other on available food source or to feed on less nutrient parts of the plant, which affect adversely the fecundity and reproductive rate of aphids [35].

In summary, this study showed that the vegetative growth of aphid-free faba bean varieties varied considerably. Aphid infestation induced an obvious injury to *V. faba* plants. There was no immune variety among test faba bean varieties, but the magnitude of damage was greatest on *V. faba major* followed by Aquadulce, whereas other varieties proved a moderate tolerance to aphid attack. Therefore, none of these varieties could be recommended to introduce into a breeding program for plant resistance towards the black bean aphid. However, introduction of more tolerant variety into agro-ecosystem leads often to a reduction in the pesticide application frequency and, therefore, the risk of pesticide use is minimized. Although, the basic information clarified in this study indicated that a further screening for *A. fabae* resistance among other genotypes, varieties and lines is worthwhile.

REFERENCES

1. Saxena, M.C., S. Weigand and L. Li-Juan, 1993. Faba bean production and research in China. In: Saxena, M.C., N.P.C. Johsen and S.N.S. Him (Eds.). Selection of Drought and Salinity Tolerance in Cool-Season Food Legumes. Breeding of Stress Tolerance. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, pp: 245-290.
2. Link, W., W. Ederer, P. Metz, H. Buiel and A.E. Melchinger, 1994. Genotypic and environmental variation for degree of cross-fertilization in faba bean. *Crop Sci.*, 34: 960-964.
3. Chapman, R. and L.P. Carter, 1976. *Crop Production: Principles and Practices*. W.H. Freeman Company, San Francisco, USA, pp: 99-109.
4. Duke, J.A., 1981. *Handbook of Legumes of World Economic Importance*. Plenum Press, New York, pp: 199-265.
5. Peoples, M.B., D.F. Herridge and J.K. Ladha, 1995. Enhancing legume N₂ fixation through plant and soil management. *Plant and Soil*, 174: 83-101.
6. Anon, 1995. *The Annual Report*, Ministry of Agriculture, Statistics Department, Amman, Jordan, pp: 15.
7. Ahmad, H.S., 1990. Water relation of faba, chickpea and lentil. *The Role of Legumes in Farming System of Mediterranean Areas*, Kluwer-Academic, Dordrecht, Netherland, pp: 96-105.
8. Hurej, M. and W. van der Werf, 1993. The influence of black bean aphid, *Aphis fabae* Scop. and its honeydew on the photosynthesis of sugarbeet. *Ann. Applied Biol.*, 122: 189-200.
9. Bond, A. and H.J.B. Lowe, 1975. Tests for resistance to *Aphis fabae* in field beans (*Vicia faba*). *Ann. Applied Biol.*, 81: 21-32.
10. Holt, J., 1980. Antibiotic resistance to *Aphis faba* in *Vicia faba* cultivars. *Ann. Applied Biol.*, 97: 66-67.
11. Holt, J. and S.D. Wratten, 1986. Components of resistance to *Aphis fabae* in faba bean cultivars. *Entomologia Experimentalis et Applicata*, 40: 35-40.
12. Bishara, S., G. Defrowy, S. Khalil and S. Weigand, 1989. *Annual Report of the Food Legume Improvement Program for 1989*, ICARDA, Aleppo, Syria, pp: 221-223.
13. Prüter, C. and C.P.W. Zebitz, 1991. Effect of *Aphis fabae* and *Uromyces vicia-favae* on the growth of a susceptible and an aphid resistant cultivar of *Vicia faba*. *Ann. Applied Biol.*, 119: 215-226.
14. Georghiou, G.P. and C.F. Taylor, 1977. Pest Resistance as an Evolutionary Phenomenon. *Proceedings XV International Congress Entomology*, Washington DC, pp: 75-785.
15. Ishag, H.M., 1973. Physiology of seed yield in field beans (*Vicia faba* L.). II. Dry matter components. *J. Agric. Sci. Cambridge*, 80: 191-199.

16. Lawes, D.A., D.A. Bond and M.H. Poulsen, 1983. Classification, origin, breeding methods and objectives. In: Hebblethwaite, P.D. (Ed.), *The Faba Bean (Vicia faba L.)*, A Basis for Improvement. Butterworths, London, pp: 23-76.
17. Hawkins, C.D.B., M.I. Aston and M.I. Whitecross, 1985. Aphid-induced changes in growth indices of three leguminous plants: unrestricted infestation. *Can. J. Bot.*, 63: 454-2459.
18. Radcliffe, E.B. and F.I. Lauer, 1970. Further studies on resistance to green peach aphid and potato aphid in the wild tuber-bearing *Solanum* species. *J. Econ. Entomol.*, 63: 110-114.
19. Wilkinson, T.L. and A.E. Douglas, 2003. Phloem amino acids and the host plant range of the polyphagous aphid, *Aphis fabae*. *Entomologia Experimentalis et Applicata*, 106: 103-113.
20. Davis, J.A., E.B. Radcliffe, D.W. Ragsdale and C.A. Thrill, 2005. Identifying and mapping mechanisms of host plant resistance to aphids in potato. The 2005 ESA Annual Meeting and Exhibition, December, 15-18, 2005, Ft. Lauderdale, FL.
21. Poehling, M.M. and Y. Morvan, 1984. Untersuchungen zur Entwicklung von *Aphis fabae* on Resistenten und Anfälligen Sorten von *Vicia faba*. *Mededelingen Rijksfaculteit Landbouwwetenschappen Gent.*, 49: 793-802.
22. Lawrence, L., 2001. Research on track to grow aphid resistant lupins. *Farming Ahead*, 114: 48-52.
23. Cichocka, E., B. Leszczynski, A.P. Ciepiela and W. Goszczycki, 2002. Response of *Aphis fabae* Scop. To different broad bean cultivars. *Elect. J. Polish Agric. Universities. Series Horticulture*, 5: 1-8.
24. Younis, M.A., M.K. Abd and R.F. Hamoudi, 1985. Effect of variety differences on growth, reproductive and survival of black bean aphids, *Aphis fabae* Scop. (Homoptera: Aphididae) in Iraq. *Zanco*, 3: 186-196.
25. Holt, J. and N. Birch, 1984. Taxonomy, evolution and domestication of *Vicia faba* in relation to aphid resistance. *Ann. Applied Biol.*, 105: 547-556.
26. Czesak, M.E., M.J. Knee, R.G. Gale, S.D. Bodach and R.S. Fritz, 2004. Genetic architecture of resistance to aphids and mites in a willow hybrid system. *Heredity*, 93: 619-626.
27. Way, M.J., 1974. Population structure in aphid colonies. In: Lowe, O.A., (Eds.), *Prespective in Aphid Biology*, Canberra, 14th Intl. Cong. Entomol., pp: 76-84.
28. Mallott, P.G. and A.J. Davy, 1978. Analysis of effects of the bird cherry-oat aphid on the growth of barley; unrestricted infestation. *New Phytologist*, 80: 209-218.
29. Barlow, A. and I. Messmer, 1982. Pea aphid (Homoptera: Aphididae) induced changes in some growth rates of pea plants. *J. Econ. Entomol.*, 75: 765-768.
30. Barlow, C.A., P.A. Randolph and J.C. Randolph, 1977. Effects of pea aphid, *Acyrtosiphon pisum* (Homoptera: Aphididae), on growth and productivity of pea plants, *Pisum sativum*. *Can. Entomol.*, 109: 1491-1502.
31. Miles, P.W., 1987. Feeding process of Aphidoidea in relation to effects on their food plants. In: Minks, A.K. and P. Harrewijn (Eds.). *Aphids: their Biology, Natural Enemies and Control*, Amsterdam, Elsevier Science Publishers B.V., pp: 321-339.
32. Vereijken, P.H., 1979. Feeding and multiplication of three cereal aphid species and their effect on yield of winter wheat. *Agricultural Research Reports*, 888, Wageningen, Pudo, pp: 58.
33. Cammell, M.E., 1981. The black bean aphid, *Aphis fabae*. *Biologist*, 28: 247-258.
34. Day, K.R., 1984. The growth and decline of a population of spruce aphid, *Elatobium abietinum*, during a three year study and the changing pattern of fecundity, recruitment and alary polymorphism in Northern Irland forest. *Oecologia* 64: 118-124.
35. Kennedy, J.S. and H.L.G. Stroyan, 1959. Biology of aphids. *Ann. Rev. Entomol.*, 4: 155-174.

Influence of *Tithonia diversifolia* Leaf Mulch and Fertilizer Application on the Growth and Yield of Potted Tomato Plants

¹M.O. Liasu and ²Abdul Kabir Khan Achakzai

¹Department of Pure and Applied Biology,
Ladoke Akintola University of Technology, Ogbomoso, Nigeria
²Department of Botany, University of Balochistan, Quetta, Pakistan

Abstract: The influence of *Tithonia* (*Tithonia diversifolia* A. Gray) leaf mulch and fertilizer application on the growth and yield of tomato seedling (*Lycopersicon esculentum* Mill.) was studied in a pot experiment. The *Tithonia* mulch and fertilizer (viz., N:P:K @ 15:15:15) application arranged in factorial combination to give four treatments. The growth and development of the tomato plants within each treatment were monitored over six weeks. Mulching with *Tithonia diversifolia* leaves and fertilizer application together promoted growth and development i.e. number of nodes, number of leaves and height, as well as fruit production i.e. number of fruits, number of seeds per fruit, fruit size, fruit shape and duration of fruiting activity more than all other treatment combinations. Tomato plants grown on soil without mulch and fertilizer gave the lowest growth and yield response. The uniqueness of *Tithonia* leaf mulch as a source of added nutrient supply to tomato plant and its antagonism to soil organisms (pests and pathogens) being the probable reason for its positive influence on tomato growth and development is discussed.

Key words: *Tithonia diversifolia* % mulch % NPK fertilizer % tomato % blossom end shapes

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) has its origin in Central America. It was domesticated in Mexico and from where it spread to the rest of the world [1]. Tomato is a seasonal; weak stemmed climbing plant of the family solanaceae. Tomatoes are warm season plants and they grow best in well-drained, fertile soil with good moisture retention capacity and having a relatively high level of organic matter. Tomato plants possess both of horticultural and agricultural importance. Though extensively cultivated as a salad vegetable, it is also grown on extensive areas for the production of soup, juice and canned tomatoes [2]. The commonly cultivated varieties in south-western Nigeria are Ibadan local and Roma.

In Nigeria, the fruits are frequently ground and used as condiments in soups and local dishes such as muke and moinmoin. As a result of import restrictions imposed in 1969 on foreign canned tomatoes and their consequent high prices, tomato cultivation increased and the price of fresh tomatoes rose up sharply. A blend of tomatoes and

hot peppers had since been canned in Nigeria and marketed under the name tomapep.

In spite of the great achievements in tomato breeding, most of the existing genetic variability among and within *Lycopersicon* spp as reported by Reid [3] is still under-exploited by tomato breeders and its more intensive utilization may allow new objectives to be reached in the future. The fruit, a berry varies considerably in size, shape, fleshiness of the mesocarp and number of seeds per fruit. The potential for extending the duration of fruiting period in order to improve yield output lies generally on genetic quality of plant but most importantly on soil conditions as early growth termination in tomato is often caused by soil nutrient depletion and root infection resulting from build up of soil pathogens.

Fertilizer is any material used on the soil to increase soil fertility. It may be chemical i.e. inorganic compound or single chemical fertilizer, or organic i.e. fertilizer that can be derived from organic matter such as animal waste or plant material e.g. green manure. Examples of chemical fertilizers include sulphate fertilizer, compound fertilizer (NPK) and ammonia fertilizer.

Mulch is a layer of material on the surface of the soil used to keep soil moist or to serve a wide variety of other purposes [4]. Organic mulches are those derived from dead plant and animal tissues, which apart from soil protection also serve as nutrient sources when they decay. Fertilizer application is more effective when applied to mulched soil than bare soil. According to Dupriez and De-Leener [5] when soil-feeding crops is rich in organic nutrients such as those derived from mulch; cultivated plants are often hardier and healthier than when nutrients come to them straight from factory made minerals. Recently, Osundina and Liasu [6] had found that soils supplemented with organic fertilizer in combination with mycorrhizae inoculation promoted growth and development of tomato better than inoculated soils combined with chemically derived fertilizers. *Tithonia diversifolia* originated in Mexico, but is now widely distributed throughout the humid and sub-humid tropics in Central and South America, Asia and Africa. Evidence suggests that *Tithonia* has been used for a wide variety of purposes. These include fodder, poultry feed, fuel, compost, land demarcation, soil erosion control, building materials and shelter for poultry [7]. The use of *Tithonia* as an effective source of biomass for annual crops has also been reported for rice [8]. But it has been more recently reported as a nutrient source for maize in Kenya, Malawi and Zimbabwe [9]. *Tithonia diversifolia* is typically found in hedges, or as small areas of pure stands in an on-farm context, although it may also extend for large areas in pure stands on common land in less populated areas, for example in the Busia District of western Kenya. Finally, stems and leaves of *Tithonia* has been reported to contain sesquiterpene lactones e.g. tagitinins (terpene) that prevent attack by termites [10, 11] and possess antimicrobial properties. The problem with mulch as source of nutrient is the low output of minerals e.g. P and N which can be alleviated by supplementing mulch from natural sources with a small dose of fertilizer.

Not much has been documented on the effect of leaf mulch in interaction with chemical fertilizer on the growth and yield of tomato. It is believed however [4] that mulch can modify the nutrient dynamics of fertilizer to enable plant derive maximum benefits from it.

MATERIALS AND METHODS

Seed collection: Tomato seeds (*Lycopersicon esculentum*) Ibadan local variety were collected on request from National Institute For Horticultural Research (NIHORT), Ibadan, Oyo State.

Soil collection: Good (i.e. loamy) top soil from the back of the Faculty of Pure & Applied Biology was scrapped with hoe and used to fill twenty four planting bags, meant to be used later for planting. The planting bags were perforated at the bottom (about eight small holes) to permit drainage of excess water and guide against the soil being water-logged.

Preparation of nursery: Two nursery boxes constructed with planks were made at the back of the faculty and filled with the soil after which the tomato seeds were broadcasted evenly on the soil. The soil within the nursery boxes was watered before and after planting of the seed. Wetting of the nursery continued twice every day (i.e. very early in the morning before sunrise and late in the evening after sunset).

Transplanting: The tomato seedlings were allowed to grow for three weeks after which they were transplanted. Prior to transplanting, all the planting bags were filled with moistened top soil and the seedling transplanted in the evening in order to give the seedlings enough time to get acclimatized to their new environment before sunrise thus safeguarding them from transplantation shock. After establishment, the tomato seedlings in each bag were thinned to one per pot.

Fertilizer application: Twenty grams of compound (Nitrogen, Phosphorus and potassium) N:P:K @ 15:15:15 fertilizer was ring applied to twelve out of the twenty-four pots. The fertilizer was applied to the tomato plant by making node around the stem and sprinkling it along the circle already marked out and later covering it with soil. Fertilizer was applied twice in the life of the tomato plants. The first one was before bud formation and the second application was just at the beginning of flower set.

Mulching: Wild sunflower (*Tithonia diversifolia*) plant leaves were collected from a nearby hedge containing pure stands and the leaves equivalent to 0.5 tones ha⁻¹ were applied to cover the soil of each potted tomato plant as mulch. Six bags from each of fertilized and unfertilized soils were subjected to mulching leading to the establishment of six replicates of four treatments namely; fertilized mulched, fertilized unmulched, unfertilized mulched and unfertilized unmulched. The tomato plants within the four fertilizers and mulch treatments were allowed to grow for twelve weeks and growth and development monitored starting from the first week after transplantation.

Data collection: The following plant growth parameters were measured at weekly intervals in plants within all replicates of the four treatments beginning from day of transplanting: Plant height using a meter rule, number of nodes and leaves plant^{G1} were measured every week after transplantation for six weeks. The means of six replicates were computed for the data generated at each week and plotted graphically against week after transplanting. Fruit yield parameters such as fruit size, shape were recorded pictorially using a Yashica camera. The mean number of fruits plant^{G1} and the average number of seeds fruit^{G1} were determined by direct counting every week beginning from the end of the week after first ripe fruit production. Fresh weights of ripe fruits harvested at the end of each week were measured using a spring balance. At the end of the experiment the weekly fruit harvests were bulked and the mean of total fresh weight yields of each of the six replicates of each treatment were determined. Standard error of means were calculated for each treatment mean (generated from six replicates) and used to separate the mean values of one treatment from the other.

RESULTS

Tomato plants subjected to mulching and fertilization exhibited the highest plant height when compared with the other treatment combinations. Weekly increases in plant height of mulched unfertilized, unmulched fertilized and mulched unfertilized tomato were comparable (Fig. 1).

Similarly (Fig. 2), the tomato plants subjected to mulching and fertilizer application exhibited the highest number of leaves plant^{G1} than all the other plants subjected to the remaining mulch and fertilized treatments. Similar trends were observed in the weekly increases in number of nodes with tomato plants growing in mulched and fertilized soils producing more nodes plant^{G1} than the other remaining combinations. The number of nodes plant^{G1} increased sharply in the first week after planting up to the 3rd week and more gently after the 3rd week up to 6th week when the experiment stopped. Mulching irrespective of fertilizer application promoted increase in number of nodes (Fig. 3).

The number of fruits produced during the first week of fruit production was highest in mulched and fertilized tomato plants with a mean of 18 fruits plant^{G1} followed by those growing in unmulched and fertilized soils with a mean of 12 fruits plant^{G1}. In mulched but unfertilized tomato plants the mean number of the fruit is 8 while in

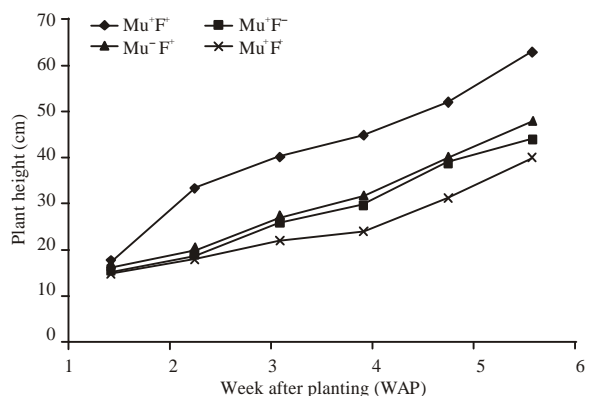


Fig. 1: The effect of *Tithonia diversifolia* leaf mulch and fertilizer (NPK) application on weekly increase in height of potted tomato plants

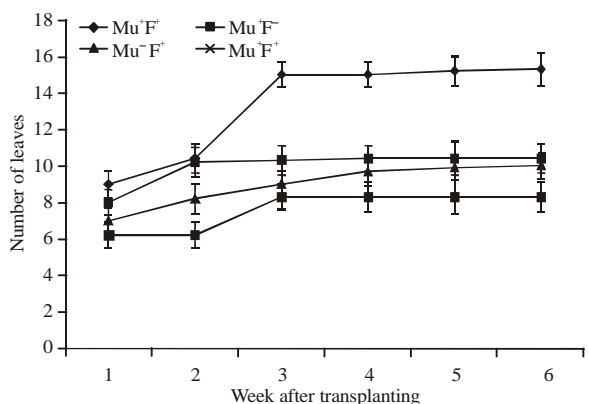


Fig. 2: Effect of *Tithonia diversifolia* leaf mulch and fertilizer (NPK) application on weekly increase in number of leaves of potted tomato plants

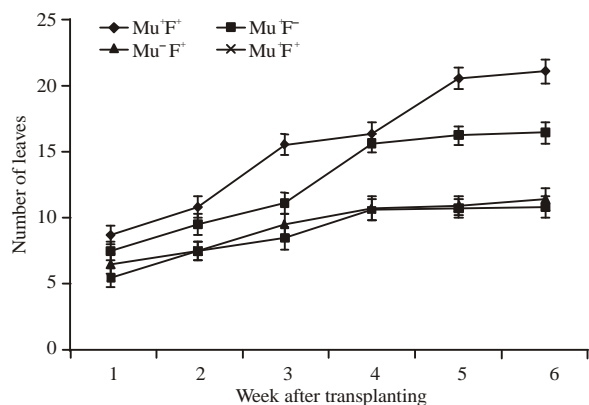


Fig. 3: Effect of *Tithonia diversifolia* leaf mulch and fertilizer (NPK) application on number of nodes

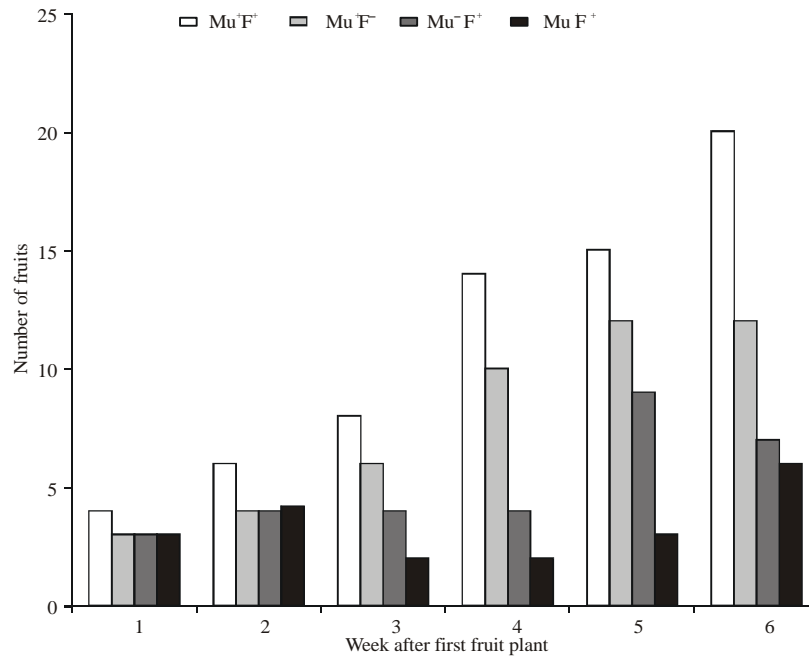


Fig. 4: Effect of *Tithonia diversifolia* leaf mulch and fertilizer (NPK) on the weekly harvests for fruit at different weeks after first fruit production Mu⁺ F⁺ Mulched and fertilized, MuG F⁺ Unmulched Fertilized, Mu⁺ FG Mulched, Unfertilized, MuG FG Unmulched, unfertilized

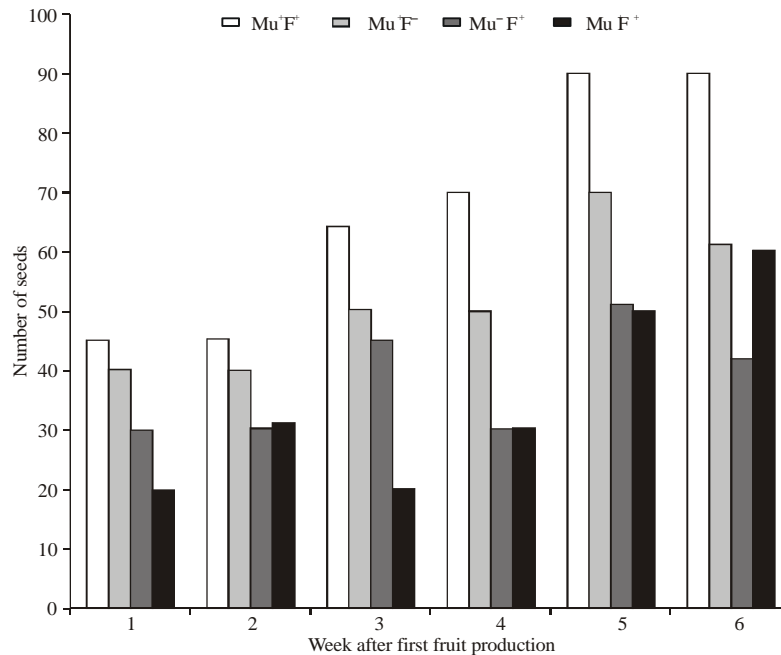
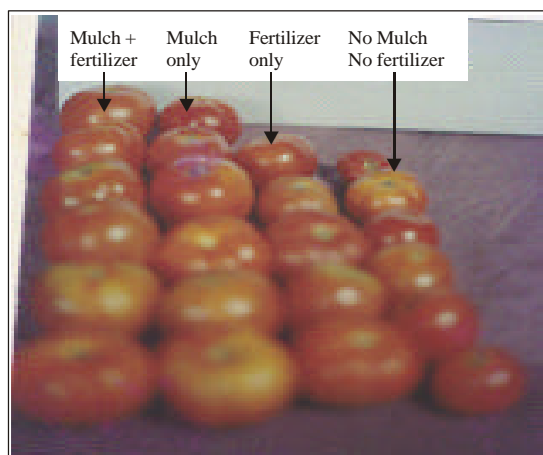
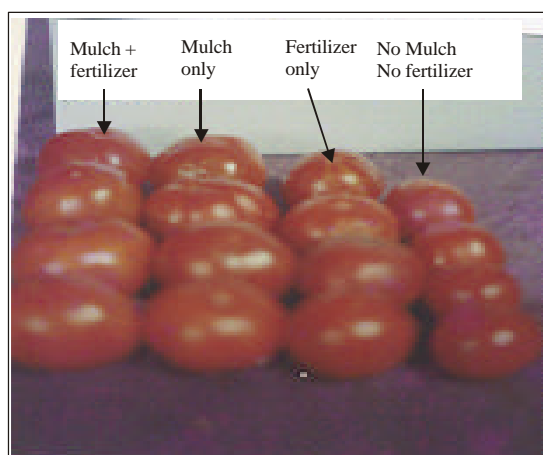


Fig. 5: Effect of *Tithonia diversifolia* leaf mulch and fertilizer (NPK) on number of seeds per fruits at different weeks after first fruit production Mu⁺ F⁺ Mulched and fertilized, MuG F⁺ Unmulched Fertilized, Mu⁺ FG Mulched, Unfertilized, MuG FG Unmulched, unfertilized



(A)



(B)

Fig. 6: Variation in (A) blossom end shapes and (B) fruits sizes of Tomato as affected by mulching and fertilizer application

unmulched and unfertilized tomato plants, the mean number of the fruits is 6. The number of fruits plant^{G1} continued to increase in the subsequent weeks until the end of the experiment. In unmulched and fertilized tomato plants, the number of fruits produced stabilized by the 5th week after first fruit production. In mulched and unfertilized tomato plants, number of fruit plant^{G1} stagnated in the 2nd, 3rd and 4th week but increased sharply by the 5th and stagnated in the 6th week (Fig. 4).

In unmulched and unfertilized tomato plants, fruiting activity was initially low as the number of fruits produced was only substantial in the 6th week after first fruit production.

The number of seeds produced fruit^{G1} in the first week of fruit production (Fig. 5) was highest in mulched

and fertilized tomato plants i.e. the mean number of seeds fruit^{G1} was 90 in mulched and fertilized tomato plant while in unmulched and fertilized tomato plant, it was 70. In mulched and unfertilized tomato plants, the mean number of seed fruit^{G1} was 60 while in the unmulched and fertilized plants; the mean number of seed fruit^{G1} is 50.

In mulched and fertilized tomato plants, the number of seeds produced fruit^{G1} stabilized by the 5th week after first fruit production before declining by the 6th week. The pattern of weekly variations in seed production fruit^{G1} was such that in unmulched and fertilized tomato plant, the number of seed produced fruit^{G1} during the 1st, 2nd, 3rd and 4th week but increased sharply by the 5th week before declining in the 6th week.

In unmulched and fertilized tomato plants, the number of seeds fruit^{G1} increased during the 1st and 2nd week followed by a decline during the 3rd week but later increased sharply by the 5th and 6th week after the first fruit production.

Tomato plants growing in mulched and fertilized soils had the biggest sizes of fruits each with round blossom end shape while those growing in unmulched and fertilized soils had moderately sized fruits though not as big as that of mulched fertilized tomato plants. They also had round blossom end shape. Unmulched and unfertilized tomato plants had fruits with the smallest sizes and shapes (Fig. 6).

DISCUSSION

Mulch and fertilizer had complementary effect on nutrient availability to plants, because mulch when it decomposes releases nutrients and organic matter (humus) which when supplied into the soil, increase the growth of the plants. Osundina and Liasu [6] made similar observations when tomato growth response to mycorrhizal inoculation in soils amended with organic matter was compared those in soils amended with chemical (inorganic) fertilizers. Humus increase nutrient retention capacity of the soil, by increasing effective cation exchange capacity [4]. Also, the fact that mulch covers the soil thereby (i) reducing the rate removal of water from the soil surface to the atmosphere i.e. evaporation, (ii) protect the soil and its organic content from direct contact with warm air thus increasing soil microbial activity consequently encouraging decomposition is probably the reason for the high growth and yield from tomatoes grown in mulched soils. Furthermore, the application of NPK fertilizer to the tomato plant supplements the nutrient content of the soil

by making available essential elements required for improved nutrition and healthy growth of the plant.

Fertilizer and mulch together not only promoted growth and yield of tomato better than fertilizer or mulch only but also improved fruit shapes (i.e. with round blossom end shapes), fruit number and number of seeds fruit^G probably because the nature of the *Tithonia* mulch does not predispose the tomato plant to attack by soil pathogens. Generally, tomato fruit quality and particularly, rounded blossom end shapes are to a large extent determined by calcium and adequate moisture supply to the plant. *Tithonia* mulch apart from being rich in nutrients including calcium, nitrogen and phosphorus can also increase the soils moisture retaining capacity [6, 9, 12]. In general, the cover of mulch creates a favorable microclimate for the activities of soil microorganisms, which help to improve and maintain the biological and physicochemical qualities of the soil thereby improving the growth performance of tomato. That fruiting activities last much longer in plant grown in mulched and fertilized soils than other soil treatments could be attributed to the fact that cessation of growth in field grown tomatoes often result from accumulation of pest and pathogens e.g. termites, bacteria, fungi and nematodes which invade the roots and spread through the plant body causing diseases and symptoms that are terminal. Such diseases also affect fruit quality flower initiation and fruit formation leading to premature termination of fruiting and even death. *Tithonia* has been shown to contain substances that prevent infestation of termites [10, 11] and possess antibiotic qualities. The implication of this unique quality of *Tithonia* mulch is its potential of extending the lifespan of tomato plants on the field, promoting fruit production at the same time consequently increasing farmer's output.

CONCLUSIONS

Addition of mulch and NPK fertilizer to the plants has produced better and healthier growth of tomato and subsequently produced high yield. This can be attributed to the addition of nutrients derived from mulching, i.e. the organic matter and probably phyto-chemicals from the leaves of *Tithonia diversifolia* added to the soil. The phyto-chemicals may play important role in the control of termite infestations and suppression of soil pathogens. Also the combination of mulch with N:P:K @ 15:15:15 provided additional nutrient and the humic materials from decaying mulch increased the nutrient retention capacity of the soil thereby providing sustained source of macro

and micro nutrients to the tomato plants [11, 12]. Finally mulch from *Tithonia* has the potential of prolonging the physiologically active lifespan of tomato including the duration of fruit-production.

REFERENCES

1. Onwueme, I.C. and J. Sinha, 1991. Field Crop Production in Tropical Africa: Principles and Practice. C.T.A. Ed. Netherlands, pp: 233-249.
2. Stevens, M.A., A.A. Kadar and M. Albright-Holton, 1997. Intercultivar variation in composition of locular and pericarp portions of fresh market tomatoes. J. Am. Soc. Hortic. Sci., 102: 689-629.
3. Reid, J.B. and M.J. Goss, 1981. Effect of living root of different plant species on the aggregate stability of two arable soils. J. Soil Sci., 31: 521-511.
4. Muller-Samann, K.M. and J. Kotschi, 1994. Sustaining Growth: Soil Fertility Management in Tropical Smallholdings. Magraf. Verlag. Weikersheim.
5. Dupriez, J. and P. De-Leener, 1989. Land and Life: African Gardens and Orchards-Growing vegetables and fruits. C.T.A. Terres and MacMillan. Netherlands.
6. Osundina, M.A. and M.O. Liasu, 1996. Responses of tomato (*Lycopersicum esculentum* Mill.) to vesicular arbuscular mycorrhizal inoculation in soils subjected to chemical and organic fertilization. Biosci. Res. Commun., 6: 181-189.
7. Liasu, M.O. and M.O. Atayese, 1999. Phenological changes in *Tithonia diversifolia* community and its potential for soil conservation. Nig. J. Weed Sci., 12: 35-44.
8. Nagaraj, S. and B.M. Nizar, 1982. Wild sunflower as a green manure for rice in the mid country west zone. Trop. Agric., 138: 69-78.
9. Jama, B.A., C.A. Palm, R.J. Buresh, A.I. Niang, C. Gachengo, G. Nziguheba and B. Amadalo, 2000. *Tithonia diversifolia* as a green manure for soil fertility improvement in Western Kenya: A Review. Agroforestry Systems, 49: 201-221.
10. Adoyo, F., J.B. Mukalama and M. Enyola, 1997. Using *Tithonia* concoctions for termite control in Busia district. Kenya. ILEIA Newsletter, 13: 24.
11. Wanjau, S., J. Mukalama and R. Thijssen, 1997. Harvesting free fertilizer. In ILEIA Newsletter, Vol: 13.
12. Atayese, M.O. and M.O. Liasu, 2001. Arbuscular mycorrhizal fungi, weeds and earthworm interactions in the restoration of soil fertility the guinea savannah region of Nigeria. Moor J. Agric. Res., 2: 103-109.

Effects of Different Pot Mixtures on Pothos (*Epipremnum aureum* Lindl. and Andre 'Golden Pothos') Growth and Development

M. Khayyat, F. Nazari and H. Salehi

Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran

Abstract: The growth of *Epipremnum aureum* Lindl. and Andre ('Golden Pothos') plants were evaluated using different pot mixtures. Plant growth was measured by 11 parameters: freshness, leaf area, leaf number, mean root length, root number, shoot number, root fresh and dry weight, shoot fresh and dry weight and mean shoot length. Parameters such as freshness, shoot length, shoot fresh and dry weight; root fresh and dry weight and root number were higher in the media containing only coco peat. Shoot number was higher in the medium containing equal leaf-mold:sand mixture compared to the other media. Highest root length and leaf area were obtained in 1:3 peat moss/coco peat mixture. Leaf number was higher in the media containing 3:1 leaf-mold/coco peat mixture. It is concluded that these differences represent a direct effect on the rooting process and that substrate characteristics are of the utmost importance for the quality of rooted cuttings.

Key words: Pothos % leaf-mold % coco peat % peat moss % quartz-sand

INTRODUCTION

In addition to the function of endogenous physiological and morphological factors which affect root formation in cuttings [1-3] and environmental or exogenous conditions during rooting may prove critical for the quality of the cutting [4]. One of the most important exogenous factors is the physical condition at the basal portion of the cutting (e.g., use of various rooting media) [4].

Thus, optimization of a rooting-substrate for cutting production is dependent on the proper combination of the following factors: water content, air content, drainage properties, nutrient balance, pH and buffer capacity, heat balance, physical stability as well as other characteristics [5-7]. Of all these factors water and air content are complementary and are of major importance to root development and cutting establishment [4]. Since no single substrate fulfills all the above mentioned requirements, several mixes have been developed.

Combinations of various media have become especially popular in cutting production of ornamentals [4]. However, considerable differences between the quality of cuttings grown on various media combinations are evident [8, 9], depending on the plant species and on the specific environmental conditions of the nursery. Although, effects of different pot mixtures on plant

growth and development is previously investigated [10-13], there are few reports on pothos plants [14, 15].

In the present investigation, the effects of different pot mixtures on rooting characteristic of *Epipremnum aureum* Lindl. and Andre 'Golden Pothos' stem cuttings at greenhouse conditions are studied.

MATERIALS AND METHODS

Media: Twenty two pot mixtures were used for this experiment. The compositions of these media by volume were as follows:

L100: Only leaf-mold

C100: Only coco peat

P100: Only peat moss

S100: Only quartz-sand

L50C50: leaf-mold/coco peat (1:1)

L50P50: leaf-mold/peat moss (1:1)

L50S50: leaf-mold/quartz-sand (1:1)

L75C25: leaf-mold/coco peat (3:1)

L75P25: leaf-mold/peat moss (3:1)

L75S25: leaf-mold/quartz-sand (3:1)

C50P50: coco peat/peat moss (1:1)

C50S50: coco peat/quartz-sand (1:1)

P50S50: peat moss/quartz-sand (1:1)

L25C75: leaf-mold/coco peat (1:3)

P25C75: peat moss/coco peat (1:3)

- S25C75: quartz-sand/coco peat (1:3)
- S25P75: quartz-sand/peat moss (1:3)
- C25P75: coco peat/peat moss (1:3)
- L25P75: leaf-mold/peat moss (1:3)
- S75P25: quartz-sand/peat moss (3:1)
- S75C25: quartz-sand/coco peat (3:1)
- L25S75: leaf-mold/quartz-sand (1:3)

All these media were poured into 2.5 L standard pots for growing pothos.

Rooting condition: Sub-terminal stem cuttings of *Epipremnum aureum* Lindl. and Andre ('Golden Pothos') were prepared in mid-May 2006. The cuttings were 20 cm in length and consisted of 4 nodes and 2 leaves (2 basal leaves were removed). All the plants after planting were placed in a greenhouse controlled on 16°C night temperature. During summer, light intensity was reduced with shading the roof to 25 to 30 klux. Plants were "hand-watered" during first month of the experiment and were supplied with 0.25-0.75% of a complete commercial nutrient solution (Rosasol Even, containing 20/20/20 combination of NPK) in irrigating water until the end of the experiment.

Data recording and analysis: The water-holding capacity and the air space of the substrates were calculated by method of Verdonck and Gabriels [16] (Table 1). Root and shoot fresh and dry weights (after being dried in oven with the temperature of 70°C for 48 h) and leaf area were measured using Analytical single-pan balance and leaf area meter (Delta-T Devices Ltd., Burwell, Cambridge, England), respectively. All the above mentioned characteristics along with root and shoot lengths; root, leaf and shoot number were measured at the end of experiment (end of August 2006). Visual quality as the freshness parameter was recorded during the growth and development of the plants using a ranking scale of 1 to 10, 1 = not fresh and rigid shoots; 10 = ideal freshness and rigidity of the shoots. Experiments were conducted in a

Table 1: The water-holding capacity and the air space of the substrates used

Medium	Total porosity (%)	Water holding capacity (%)
Quartz-sand	96	36
Peat moss	87	56
Leaf-mold	91	68
Coco peat	85	71

Completely Randomized Design (CRD) with 22 treatments, 4 replications in each. Means were compared using Duncan's multiple range tests (DMRT) at 5% level.

RESULTS

There were significant differences between substrates regard to quality of roots produced and shoots developed. Higher root number (Fig. 1 and 2) and root fresh (Fig. 3) and dry (Fig. 4) weights were observed in the media containing only coco peat and significant differences were observed between this medium and the other pot mixtures.

Higher root length (Fig. 5) was obtained in P25C75 mixture. However, no significant differences were observed between this medium with C25S75 and C100 with C25S75 mixtures (Fig. 5). The media containing only coco peat were the best treatments according to parameters such as shoot fresh (Fig. 6) and dry (Fig. 7) weights and shoot length (Fig. 8 and 9). However, shoot length did not show any significant differences between C100 with C50P50 and C50P50 with L50P50 mixtures (Fig. 9).

Higher leaf area (Fig. 10) was observed in P25C75 compared to the other pot mixtures. However, no significant differences were shown between P25C75 with C50P50 and C100 mixtures (Fig. 10). The number of shoots produced in L50S50 was significantly more than the other media (Fig. 11).

The highest leaf number (Fig. 12) was observed in L75C25 mixture. However, no significant differences were shown between L75C25 and L100 mixtures (Fig. 12).



Fig. 1: Root production on a pothos cutting cultured in C100 medium

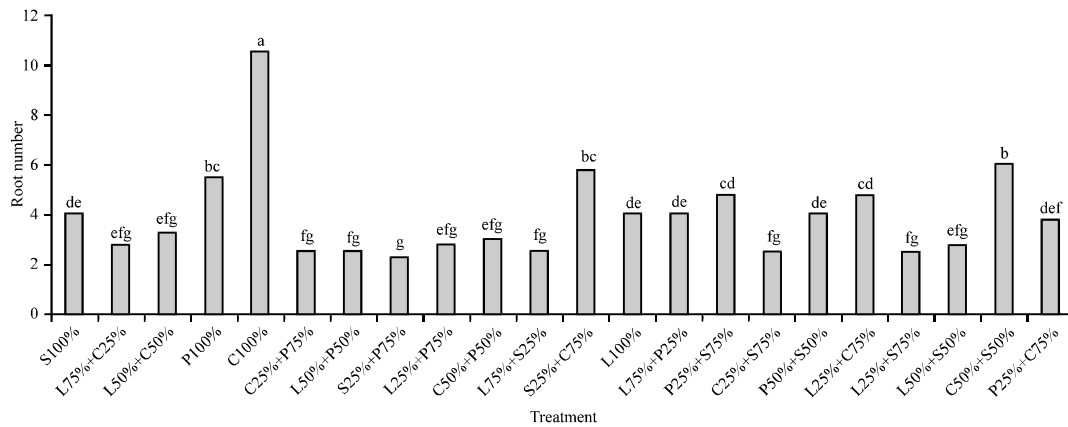


Fig. 2: Number of roots produced in each treatment. Bars with the same letters are not significantly different according to DMRT at 5% level

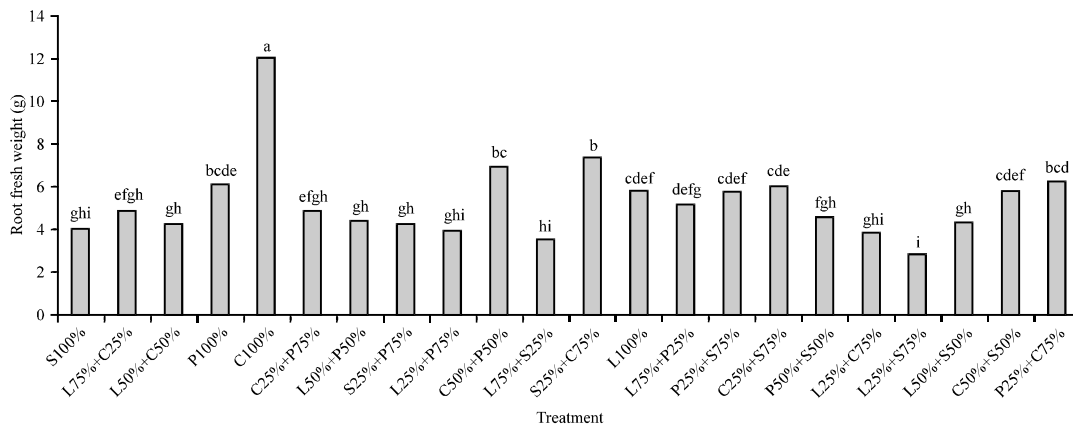


Fig. 3: Fresh weight of roots produced in each treatment. Bars with the same letters are not significantly different according to DMRT at 5% level

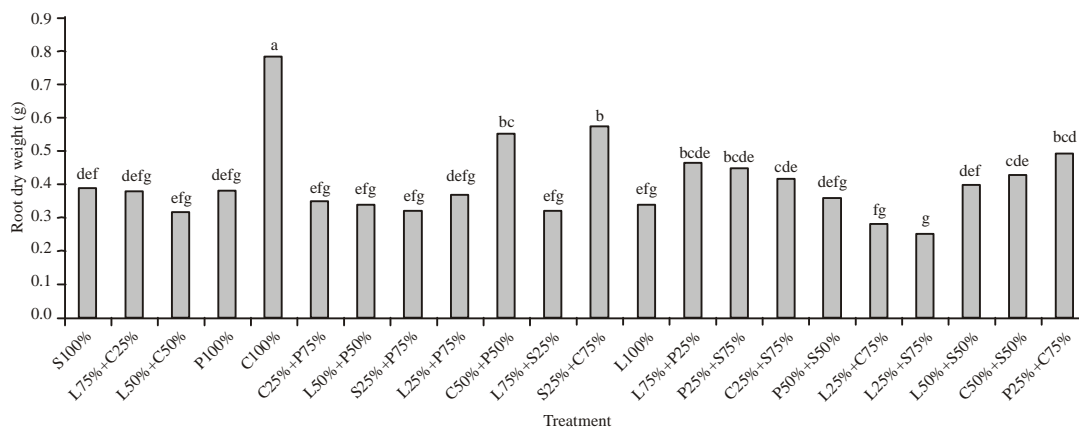


Fig. 4: Dry weight of roots produced in each treatment. Bars with the same letters are not significantly different according to DMRT at 5% level

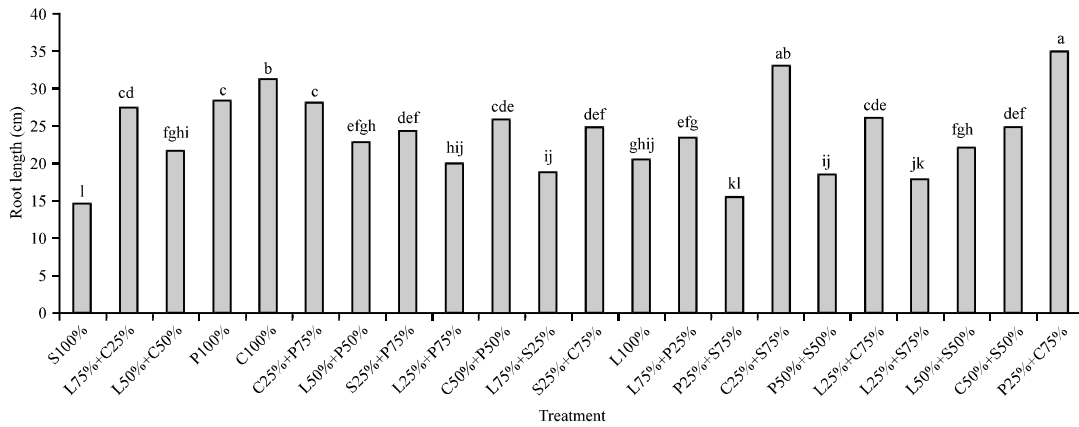


Fig. 5: The length of roots produced in each treatment. Bars with the same letters are not significantly different according to DMRT at 5% level

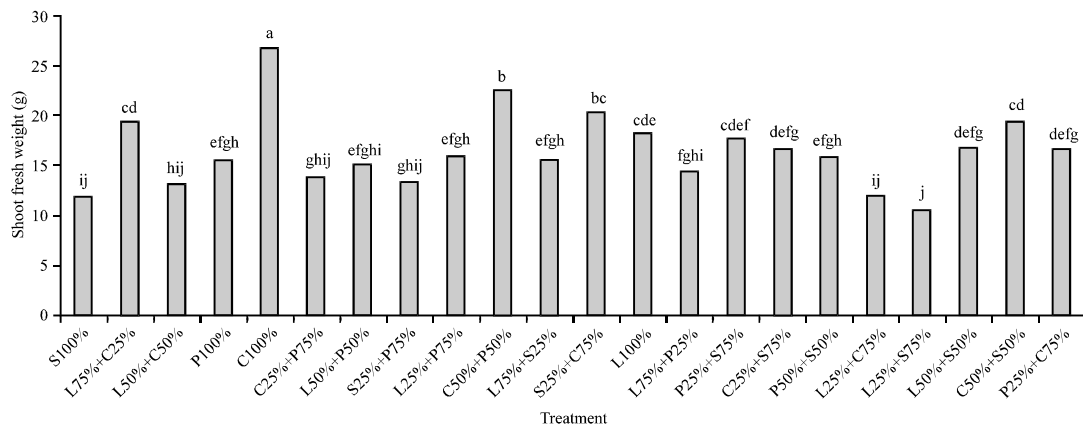


Fig. 6: Fresh weight of shoots produced in each treatment. Bars with the same letters are not significantly different according to DMRT at 5% level

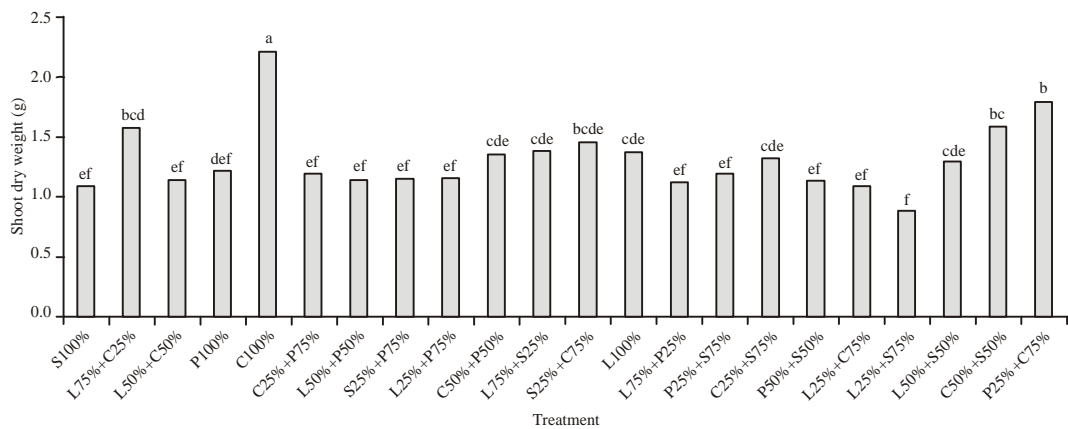


Fig. 7: Dry weight of shoots produced in each treatment. Bars with the same letters are not significantly different according to DMRT at 5% level



Fig. 8: Long shoots produced on the pothos cuttings cultured in C100 medium

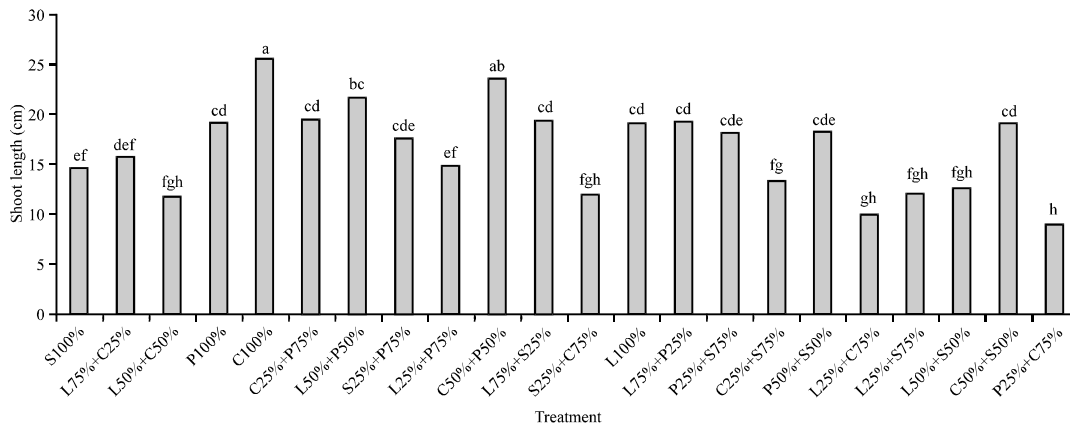


Fig. 9: The length of shoots produced in each treatment. Bars with the same letters are not significantly different according to DMRT at 5% level

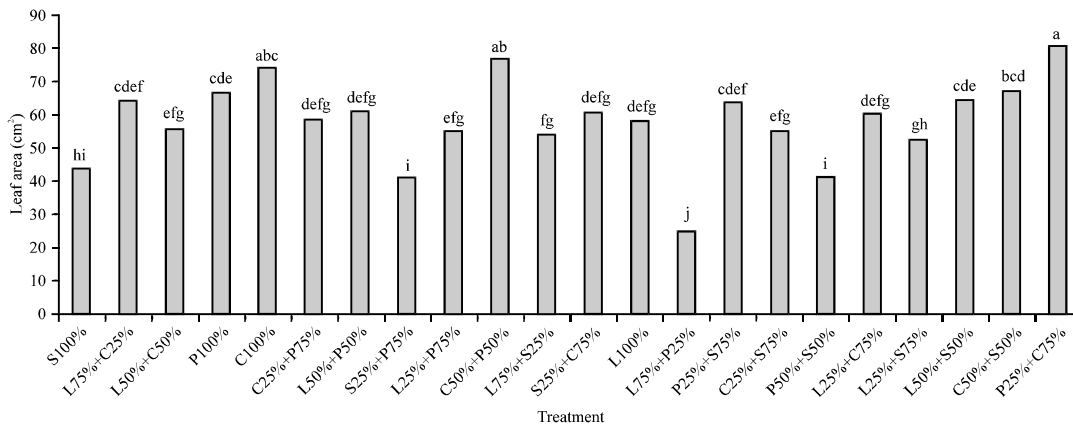


Fig. 10: Mean area of leaves produced in each treatment. Bars with the same letters are not significantly different according to DMRT at 5% level

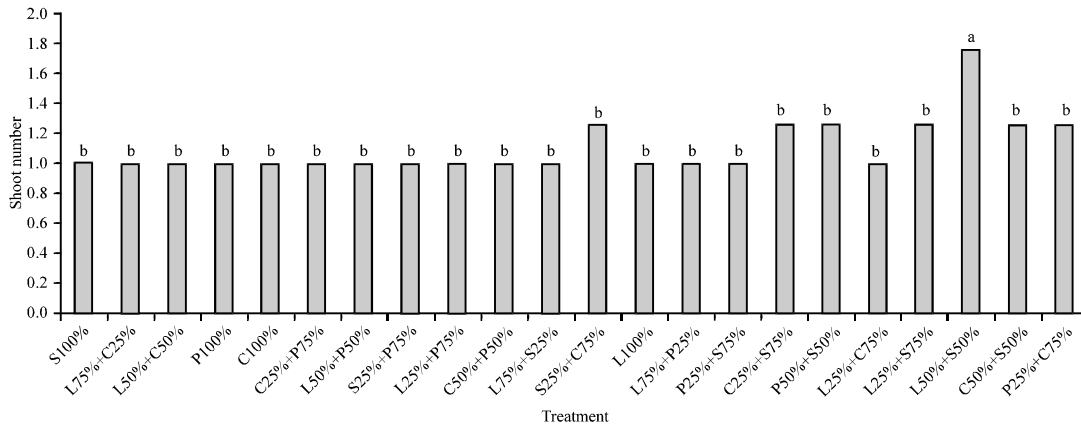


Fig. 11: Number of shoots produced in each treatment. Bars with the same letters are not significantly different according to DMRT at 5% level

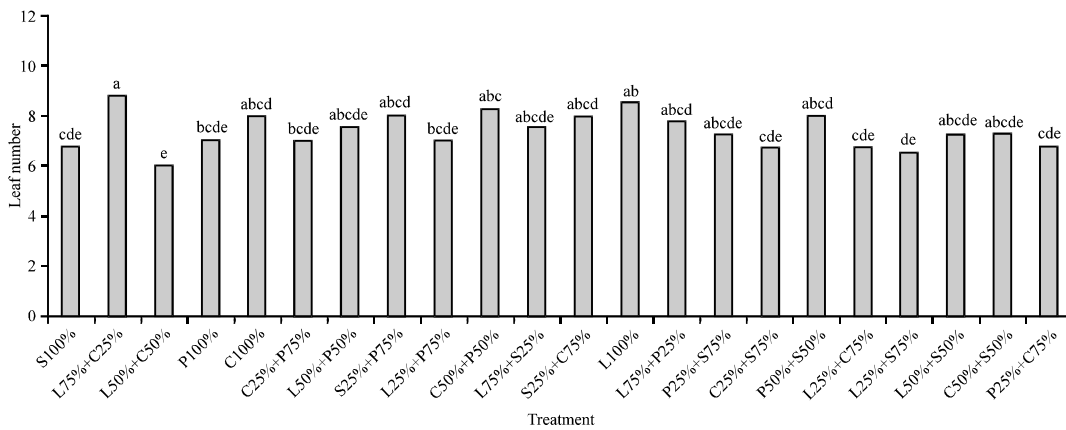


Fig. 12: Number of leaves produced in each treatment. Bars with the same letters are not significantly different according to DMRT at 5% level

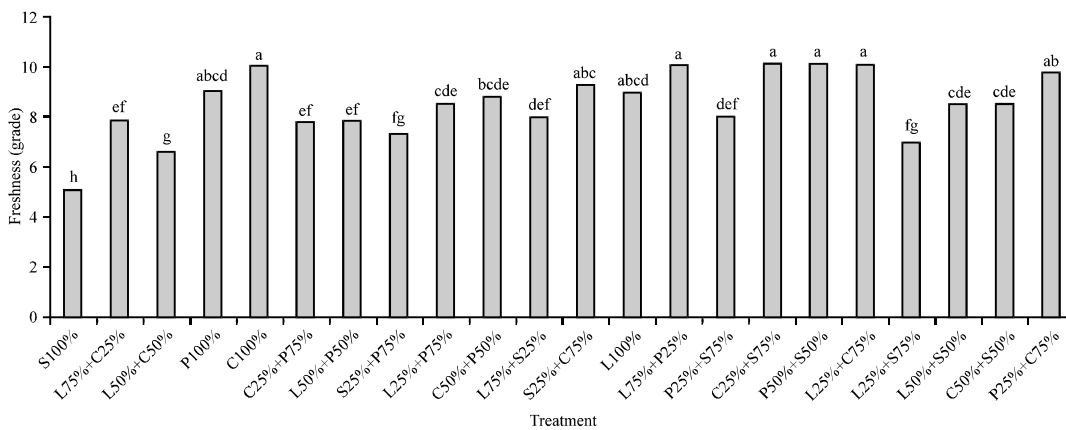


Fig. 13: Visual quality (freshness of the plants) in each treatment using a ranking scale of 1 to 10, 1 = not fresh and rigid shoots; 10 = ideal freshness and rigidity of the shoots. Bars with the same letters are not significantly different according to DMRT at 5% level

Regard to the freshness of plants, media containing C100, L75P25, C25S75 and L25C75 mixtures were the best treatments compared to all of the other treatments (Fig. 13).

DISCUSSION

There were significant differences along with media efficiency. Differences in performance on various rooting media can be attributed to a direct effect of the substrate on the basal portion of the cutting, rather than to indirect or earlier physiological changes. The large differences in the quality of root system and shoot characteristics do indeed indicate the importance of direct effect of the media.

Improved root formation and growth on C100 and P25C75 mixtures might be related to the better aeration and drainage conditions and water maintenance capability of these substrates compared to the other media [16-18] which are critical for the first phase of the root initiation.

The presence of the leaves on the cuttings may reflect earlier growth of the root system, but the other environmental factors can also be involved. Thus, while new leaf development on C100 and P25C75 mixtures largely agrees with the superior root development on these media, P100 has less deleterious effects on leaf growth.

On the other hand, sand mixtures allowed moderate leaf development, although root growth was too low. In the media containing leaf-mold, high leaf development was obtained, although root growth was low. Since these phenomena cannot be explained solely by differences in the water/air relationship of the various rooting media, other factors are probably involved. Mechanical impedance and reduced porosity is one such factor which may restrict root formation [19].

REFERENCES

1. Altman, A., 1972. The Role of Auxin in Root Initiation in Cutting. Proceedings of the International Plant Propagators Society, pp: 280-294.
2. Haissig, B.E., 1974. Influence of auxin and auxin synergists on adventitious root primordium initiation and development. New Zealand J. For. Sci., 4: 311-323.
3. Hartman, H.T., D.E. Kester, F.T. Davies and R.L. Geneve, 2002. Plant Propagation, Principles and Practices. 7th Edn. Prentice Hall Incorporation.
4. Altman, A. and D. Freudenberg, 1983. Quality of *Pelargonium graveolens* cutting as affected by the rooting medium. Sci. Hortic., 19: 379-385.
5. De Boodt, M. and O. Verdonck, 1972. The physical properties of the substrates in horticulture. Acta Hortic., 26: 37-44.
6. Bunt, A.C., 1976. Modern Potting Compost. George Allen and Unwin Press.
7. Goh, K.M. and R.J. Haynes, 1977. Evaluation of potting media for commercial nursery production of container grown plants. New Zealand J. Agric. Res., 20: 363-370.
8. Poole, R.T. and W.E. Waters, 1972. Evaluation of Various Potting Media for Growth of Foliage Plants. Proceedings of Florida State Horticultural Society, pp: 395-398.
9. Conover, C.A. and R.T. Poole, 1974. Influence of Media and Fertilizer Rates on *Aglaonema* 'Fronsher'. Proceedings of Florida State Horticultural Society, pp: 177-183.
10. Nowak, J.S. and Z. Strojny, 2003. Effect of different container media on the growth of gerbera. Acta Hortic., 608: 59-63.
11. Nikolova, N., I. Konczak and V. Kolarov, 1985. Development of tight greenhouse rose buds into flowers on an artificial medium. Acta Hortic., 167: 435-440.
12. Samartzidis, C., T. Awada, E. Maloupa, K. Radoglou and H.-I.A. Constantinidou, 2005. Rose productivity and physiological responses to different substrates for soil-less culture. Scientia Hortic., 106: 203-212.
13. Douglas, M.H., B.M. Smallfield, G.A. Parmenter, L.C. Burton and A.J. Heaney, 2000. Effect of growing media on the production of ginseng (*Panax ginseng*) in Central Otago, New Zealand. New Zealand J. Crop and Hortic. Sci., 28: 195-207.
14. Agut, A., 1984. Response of pothos in ten greenhouse media. Acta Hortic., 150: 247-254.
15. Sameei, L., A. Khalighi, M. Kafi and S. Samavat, 2004. Peat moss substituting with some organic wastes in pothos (*Epipremnum aureum* cv. Golden Pothos) growing media. Ir. J. Hortic. Sci. Technol., 6: 79-88.
16. Verdonck, O. and R. Gabriels, 1992. I. Reference method for the determination of physical properties of plant substrates. II. Reference method for the determination of chemical properties of plant substrates. Acta Hortic., 302: 169-179.

17. Eleni, M., K. Sabri and Z. Dimitra, 2001. Effect of growing media on the production and quality of two rose varieties. *Acta Hortic.*, 548: 79-83.
18. Noguera, P., M. Abad, V. Noguera, R. Puchades and A. Maqueira, 2000. Coconut coir waste, a new and viable ecologically friendly peat substitute. *Acta Hortic.*, 517: 279-286.
19. Nicolosi, R.T. and T.A. Fretz, 1980. Evaluation of root growth in varying medium densities and through dissimilar soil surfaces. *HortSci.*, 15: 642-644.

Irrigation Scheduling for Green Pepper (*Capsicum annuum* L.) Grown in Field Conditions by Using Class - A Pan Evaporation Values

¹A. Ertek, ²S. Öensoy, ³Ğ. Gedik and ³C. Küçükyumuk

¹Department of Irrigation, Faculty of Agriculture, Süleyman Demirel University, 32260, Isparta, Turkey

²Department of Horticulture, ³Department of Irrigation, Yüzüncü Yıl University, 65080, Van, Turkey

Abstract: This study was conducted to determine the most suitable amount of applied water and the interval of irrigation water for green pepper plants by using the pan evaporation values in field conditions. Irrigation water was applied based on cumulative class-A pan evaporation within the irrigation intervals. Irrigation treatments consisted of two irrigation intervals based on pan evaporation (I1: 25 ± 5 mm E_{pan} ; I2: 50 ± 5 mm E_{pan}) and three plant-pan coefficients (K_{cp} 1: based on percent crop canopy closure; K_{cp} 2: 0.75 and K_{cp} 3: 1.10). According to the results, the average irrigation water values of treatments varied from 233 to 783 mm; the average evapotranspiration values of treatments ranged from 263 to 711 mm; and the green green pepper fruit yield ranged from 5.41 to 16.85 t ha⁻¹. Furthermore, K_{cp} 3 treatment that irrigated with the highest amount of water gave the highest early fruit yield and the highest total fruit yield was obtained from I1 K_{cp} 3 treatment. Yield response factor (K_y) was determined as 0.91. E_t/E_{pan} ratios of the treatments varied from 0.34 to 1.76. In addition, it was determined that irrigation programs significantly affected the yield ($p < 0.001$). Moreover, significant positive linear correlation ($p < 0.01$) between irrigation water amount and plant vegetative growth traits and between plant water consumption and the fruit yield were determined. Thus, irrigation interval at 50 ± 5 mm E_{pan} and K_{cp} 3 plant-pan coefficient could be recommended for green pepper irrigation to save labor cost and time.

Key words: Water use efficiency % green pepper evapotranspiration % irrigation scheduling

INTRODUCTION

The typical purpose of irrigation is to favorably maintain the water status of plants. It, therefore, seems normal that irrigation should be accurately scheduled by using some measures of plant water status [1]. It is also important to know the water susceptibility of plants for suitable irrigation management [2].

Adequate amount of water must be applied at the right time in order to get higher crop yield in irrigated lands. Therefore, it is vital to determine the water consumption of plants and periods that plants are susceptible for water beside the irrigation intervals in order to increase crop yield in a limited area. Water requirement of plants from seed sowing to the harvest varies depending on plant species and plant growth stages. Excessive irrigation just after transplanting may cause coarse, tall, but weak growth, small inflorescences or flower shedding and small fruits in plants.

The world production of fresh fruit green pepper is about 24 million tons from 1.66 million ha and its

production in Turkey is about 1.79 million tons from 88.000 ha area [3]. Green peppers develop relatively shallow root systems, to a depth of about 60 cm. They require about 25-50 mm of rainfall or irrigation per week for optimum production. Drought stress during early growth stages might most probably reduce plant size and cause blossom shed and reduced fruitset [4]. Therefore, irrigation and water management become very critical for green pepper. Green pepper plants have shallow root systems; they, therefore, cannot tolerate to drought. The need for water is especially high during the flowering and fruit setting. Fields should be irrigated if there are signs of wilting at midday. Green pepper plants are also sensitive to water logging. Flooded fields should be drained within 48 h. Otherwise, the green pepper plants may soon die. Furrow or drip irrigation is recommended. Sprinkler irrigation should be avoided as wet leaves and fruit promote disease development [5].

Pan evaporation is a method widely used to schedule irrigation because of its easy application and inexpensive to use [6, 7]. With available pan coefficient in hand, pan

evaporation method can be used in the arrangement of irrigation programs. Therefore, evapotranspiration of growing plants can be estimated by using pre-determined coefficients and pan evaporation method [8].

The aim of this study was to determine the most suitable irrigation schedule for green pepper plants grown in the field conditions by using class-A pan evaporation and related plant-pan coefficients.

MATERIALS AND METHODS

This study was carried out in a farmer's field located in Central Van, in 2001 (between 35° 55' and 39° 24'N latitude and 42° 05' and 44° 22'E longitude and 1725 m altitude). The continental temperate climate rules over the region; while the highest average temperature is in July (22.1°C), the lowest is in January (-3.7°C); average wind speed is 2.3 m s⁻¹; precipitation is insufficient in summers when plant water use is greatest.

The soil at the study site is loamy and almost flat. Some soil characteristics related with irrigation are seen in Table 1. One month-old seedlings of green pepper cultivar Demre, which is one of the most important cultivars produced in Turkey with long and thin fruit, were transplanted in 80x30 cm spacing on May 27th, 2001 and adequately watered. The distance between the plots, which consisted of four rowed 24 plants in 5.76 m² was 100 cm. Diamonium phosphate (125 g DAP) was applied to each plot before transplanting the seedlings and 50 g urea as a nitrogen source per plot was given both at initial flowering (July 7th) and at initial fruit maturation stages (August 6th). During the growing season, plant protection measures and hoeing were practiced to the plots. Plants were hoed in order to both break the soil crust and fight against the weeds.

Irrigation water (2 l s⁻¹) was supplied from a well by a pump. Furrows in each plot were irrigated by a hose (4 cm in diameter) with a flow meter on it. Water is in C₃S₁ class (sodium risk is low; EC is medium) and it can be used for irrigation.

Treatments consist of two different irrigation intervals based on pan evaporation (I1: 25±5 mm E_{pan}; I2: 50±5 mm E_{pan}) and three different plant-pan coefficients (K_{cp}1: based on percent crop canopy closure; K_{cp}2: 0.75

and K_{cp}3: 1.10). Treatments were arranged in a Completely Randomized Block Design with three replications. Irrigation was done in short blunt furrows. Plots were irrigated up to field capacity one week after the transplanting. Then, scheduled irrigation was initiated when cumulative pan evaporation, values reached 25±5 mm or 50±5 mm. Evaporation between the irrigation intervals was measured with a Class-A pan located nearby to the plots.

In calculation of irrigation water amount, class-A pan evaporation whose fundamentals are given in the articles of Doorenbos and Pruitt [8]; Kanber [9] were used (Eq. 1):

$$I_r = E_{pan} * K_{cp} \quad (1)$$

Where I_r is the amount of applied irrigation water (mm), E_{pan} is the evaporation at Class-A pan (25±5 mm or 50±5 mm) and K_{cp} is the plant-pan coefficient. Eq. (2) was used in the determination of K_{cp}1 according to plant coverage.

$$K_{cp}1 = (W_p/W_b) \times 100 \quad (2)$$

Where W_p is the width of plant canopy (cm) and W_b is the bed spacing (cm).

E_t was calculated for each treatment by a water balance method (Equation 3) [10].

$$E_t = I_r + P + C_r - D_p - R_r + \Delta s \quad (3)$$

Where; E_t: evapotranspiration (mm), I_r: irrigation water (mm) calculated in Equation 1 for each treatment, p: precipitation (mm), C_r: capillary rise (mm), D_p: loss by deep percolation (mm), R_r: surface run-off (mm), Δs: change in profile soil water content (mm).

Precipitation (P) was measured daily at a nearby weather station. C_r was considered as zero because there was no high underground water problem in the area. If available water in the root zone (90 cm) and total amount of applied water by irrigation were above the field capacity, it would be assumed that mentioned water leaked and called as the deep percolation value [11].

Table 1: Soil characteristics of trial plots

Depth (cm)	(g cm ⁻³)	FC (P _w)	WP (P _w)	Saturation (%)	pH	EC (dS m ⁻¹)	Salt (%)	Lime (%)	P (kg ha ⁻¹)	K (kg ha ⁻¹)	Organic matter (%)	Structure
0-30	1.42	14.93	7.95	41.0	7.94	2.91	0.08	4.69	3.89	36.3	0.95	Loamy
30-60	1.50	14.11	7.59	39.0	8.01	3.06	0.08	6.86	3.89	31.6	0.83	Loamy
60-90	1.44	18.23	9.94	41.6	8.06	2.33	0.06	10.56	0.46	29.9	0.70	Loamy

(: Unit weight of soil; FC: Field Capacity; WP: Wilting Point

Soil water measurements were taken throughout the crop growth season. Profile soil water contents up to the 90 cm depth in 30 cm increments were measured gravimetrically (oven dry basis) at transplanting, before each irrigations and final harvest.

Irrigation Water Use Efficiency (IWUE) and water use efficiency (WUE) was calculated with Eqs. (4 & 5) [12, 13].

$$IWUE = (E_y/I_r) \times 100 \quad (4)$$

$$WUE = (E_y/E_t) \times 100 \quad (5)$$

where; IWUE: irrigation water use efficiency (t haG¹ mm), E_y: marketable yield (t haG¹), WUE: water use efficiency (t haG¹ mm).

Moreover, Equation 6 was used to determine the contribution of different irrigation levels on plant water consumption [12, 13].

$$I_{rc} = (I_r/E_t) * 100 \quad (6)$$

Where I_{rc} is the irrigation water compensation for plant water consumption (E_t) (%).

In order to determine yield-response factor (K_y), Eq. (7) was used advised by Stewart *et al.* [14] and Doorenbos and Kassam [15]. Therefore, using Eq. 7, relative yield decrease related to per unit water deficit, can be predicted.

$$K_y = (1 - Y/Y_m) / (1 - E_t/E_{m}) \quad (7)$$

Where, Y: yield (t haG¹), Y_m: maximum yield (t haG¹), E_t: plant water consumption, (mm), E_m: maximum plant water consumption, (mm), K_y: yield-response factor. Yield-response factor (K_y), is a relative value which indicates the yield sensitivity under per unit water deficit.

Marketable green pepper were hand harvested by once a week and then weighted. Furthermore, the number, diameter and length of fruit were also determined by counting or measuring. The first four harvests were considered as the early yield. The height, coverage and stem diameter of plants were also measured and the number of lateral branches was counted.

Analysis of variance was performed on the yield data obtained from the treatments. The level of the significant difference (LSD at p<0.01) was used in the ANOVA to test the effect of treatments on different response variables [16].

RESULTS AND DISCUSSION

Applied irrigation water amount (I_r) and plant water consumption (E_t): A total 45 mm of water applied to all

treatments prior to the scheduled irrigations. Soil water deficit in all plots was replenished to the field capacity in 0-90 cm soil depth and then scheduled irrigation based on 25 and 50 mm of cumulative evaporation were initiated. During the growing season in which 671 mm of evaporation occurred, treatments with 25±5 and 50±5 mm evaporation intervals were irrigated 26 and 13 times, respectively.

While K_{cp}1 treatments applied the lowest amount of water (233 mm), K_{cp}3 treatments applied the highest amount of water (783 mm). E_t increased with the amount of applied irrigation water. While the I1K_{cp}1 treatment had the lowest E_t (263 mm), the I1K_{cp}3 treatment had the highest E_t (796 mm). Although they were watered with the same amount of water, in the frequently watered treatments, plant consumed much more water than in less frequently irrigated plants. There was a little rainfall (11 mm) during the experiment (Table 2).

FAO [3] informed that total water requirements (E_t) was 600 to 900 mm and up to 1250 mm for long growing and harvesting periods and several pickings.

Fruit yield data: The first fruit was harvested 56 days after transplanting of seedlings and there were 8 harvests during the growing season which lasted 113 days. K_{cp}3 treatments irrigated the most abundantly and having the highest water consumption gave the highest early yields in both irrigation intervals. The early yield increased as the amount of applied water increased in both irrigation intervals. This finding proposes and shows that green pepper is a highly susceptible plant to water deficit and water scarcity in early growing period decreases the early green pepper yield. The average total yields increased with greater amounts of water applied for all treatments. The highest average total yields were also obtained from the K_{cp}3 treatments in both irrigation intervals, while K_{cp}1 treatments received with the least amount of water gave the lowest yields. Throughout the harvest period, although higher yields were usually followed by relatively lower yields, there was a relative increase in yield (Table 2).

Water-yield relationships: It was determined that irrigation treatments had significant effects on the green pepper fruit yield (Table 3). While there was considerable effect of K_{cp} on yield (p<0.001), I and I * K_{cp} interaction on yield were not significant. Treatments irrigated based on the K_{cp}3 coefficient resulted in more yield than other treatments. The more water applied to the treatments the more green pepper yield was obtained.

Moreover, significant correlations were obtained (p<0.01) between yield and I_r or between yield and E_t and

Table 2: Yield components and irrigation values

Yield components and irrigation values	Treatments					
	I1K _{cp} 1	I1K _{cp} 2	I1K _{cp} 3	I2K _{cp} 1	I2K _{cp} 2	I2K _{cp} 3
Early yield, t haG ¹	1.02	1.39	1.91	0.84	1.86	1.90
Mean fruit yield, t haG ¹	5.57	11.67	16.18	5.41	13.78	16.85
Fruit number	590.00	998.00	1286.00	466.00	1094.00	1294.00
Fruit diameter, mm	12.06	14.09	13.98	11.91	14.02	13.86
Fruit length, cm	11.21	12.80	13.31	11.21	13.95	14.34
Mean fruit weight, g	5.44	6.73	7.25	6.68	7.25	7.50
Plant height, cm	34.58	42.67	44.88	37.83	48.00	48.90
Plant coverage %	37.40	53.40	54.10	38.50	58.10	62.40
Numbers of lateral branches	5.00	5.28	5.67	5.00	5.44	5.84
Stem diameter (cm)	9.67	12.45	13.00	10.72	13.39	13.59
I _r , mm	233.00	548.00	783.00	246.00	548.00	783.00
E _t , mm	263.00	581.00	796.00	268.00	565.00	711.00
IWUE, kg mG ³	2.40	2.10	2.10	2.20	2.50	2.20
WUE, kg mG ³	2.10	2.00	2.00	2.00	2.40	2.40
Irc %	88.60	94.50	98.40	91.80	97.00	100.00
Relative pepper yield %	33.10	69.30	96.00	32.10	81.80	100.00
Relative E _t %	33.00	72.90	100.00	33.60	70.90	89.20

Table 3: Mean pepper yields and fruit number of treatments compared with Duncan statistical method

Treatments	Yield		Fruit number	
	Mean (g)	Significant ranges	Mean	Significant ranges
I1	6415.9	9a	958	9a
I2	6917.6	9a	951	9a
K _{cp} 1 ***	3162.5	6c	528	6c
K _{cp} 2 ***	7327.5	6b	1046	6b
K _{cp} 3 ***	9510.2	6a	1290	6a

*** LSD.001 = 1932.53 (Yield); *** LSD.001 = 192 (fruit number)

shown in Fig. 1a. As I_r, therefore E_t, increased, yield also increased. E_t was a little bit more effective on yield (R² : 0.97 **) than I_r (R²: 0.95 **) (Table 4). These all indicate that green pepper plants are very sensitive to water deficiency. Furthermore, it was understood by visual inspection and eating that the fruit obtained from the treatments with higher K_{cp} had better quality than others. The less water applied to the treatments the more misshapen and dull colored pepper fruit was obtained. Some other studies have also shown the physiological response of green pepper plants to water stress data on the relationship between water use and yield of green pepper [17, 18].

The relationships between relative yield decrease and relative evapotranspiration deficit for the total growing period is given in Fig. 1b. Yield response factor (K_y) was determined as 0.91 and 1.00 for all growing period and the period after flowering, respectively. Thus, up to 1.00 unit

decrease in yield for each unit water deficit is expected for green pepper grown outdoor. Therefore, for high yield and quality, the crop needs a controlled supply of water throughout the growing period. FAO [3] and Sagardoy *et al.* [19] informed that the yield-response factor (K_y) was 1.1 for pepper.

In order to obtain high yield in green pepper, an adequate water supply and relatively moist soils are required during the total growing period. Reduction in water supply during the growing period in general has an adverse effect on yield and the greatest reduction in yield occurs when there is a continuous water shortage until the time of first harvest. The period at the beginning of the flowering period is the most sensitive to water shortage and soil water depletion in the root zone during this period should not exceed the 25 percent. Controlled irrigation is essential for high yield because green pepper is sensitive to both over and under irrigation [3].

Table 4: Correlation equations and coefficients (R) among mean fruit weight, fruit length, fruit diameter, fruit number, irrigation water, evapotranspiration and yield

Yield components	Yield	Irrigation water	Evapotranspiration	Fruit Number	Cover percentage	Plant lateral
branch number (PLBN)	(Y)	(I _r)	(E _t)	(FN)	(CP)	
Fruit number (FN)	Y = 0.0143 FN-2.06 R ² = 0.99 **	FN = 1.413 I _r +214.55 R ² = 0.96 **	FN = 1.5437 E _t +135.8 R ² = 0.96 **			
Fruit diameter (FD)	Y = 4.41 FD-47.17 R ² = 0.82 **	FD = 0.00371 I _r +11.37 R ² = 0.76 **	FD = 0.0042 E _t +11.09 R ² = 0.82 **			
Fruit length (FL)	Y = 3.59 FL-34.38 R ² = 0.91 **	FL = 0.00491 I _r +10.23 R ² = 0.80 **	FL = 0.0052 E _t +10.03 R ² = 0.75 **			
Mean fruit weight (MFW)	Y = 5.662 MFW-26.97 R ² = 0.69 **	MFW = 0.0025 I _r +5.51 R ² = 0.66 **	MFW = 0.0026 E _t +5.41 R ² = 0.62 **			
Plant height (PH)	Y = 0.828 PH-23.86 R ² = 0.86 **	PH = 0.0202 I _r +32.21 R ² = 0.76 **	PH = 0.022 E _t +31.39 R ² = 0.71 **	FN = 55.35 PH-1415 R ² = 0.80 **		
Cover percentage (CP)	Y = 0.464 CP-11.94 R ² = 0.90 **	CP = 0.0384 I _r +30.59 R ² = 0.82 **	CP = 0.041 E _t +28.89 R ² = 0.78 **	FN = 31.81 CP-656.9 R ² = 0.88 **		
Plant lateral branch number (PLBN)	Y = 14.23 PLBN-64.87 R ² = 0.95 **	PLBN = 0.0014 I _r +4.7 R ² = 0.95 **	PLBN = 0.0014 E _t +4.6 R ² = 0.87 **	FN = 971.2 PLBN-4262.3 R ² = 0.91 **	CP = 11.81 PLBN-14.71 R ² = 0.62 **	
Plant stem diameter (PSD)	Y = 3.01 PSD-24.94 R ² = 0.89 **	PSD = 0.0059 I _r +9.05 R ² = 0.82 **	PSD = 0.0064 E _t +8.75 R ² = 0.80 **	FN = 202.8 PSD-1506.9 R ² = 0.84 **	CP = 6.35 PSD-26.4 R ² = 0.95 **	PLBN = 0.19 PSD-3.03 R ² = 0.79 **

** p<0.01

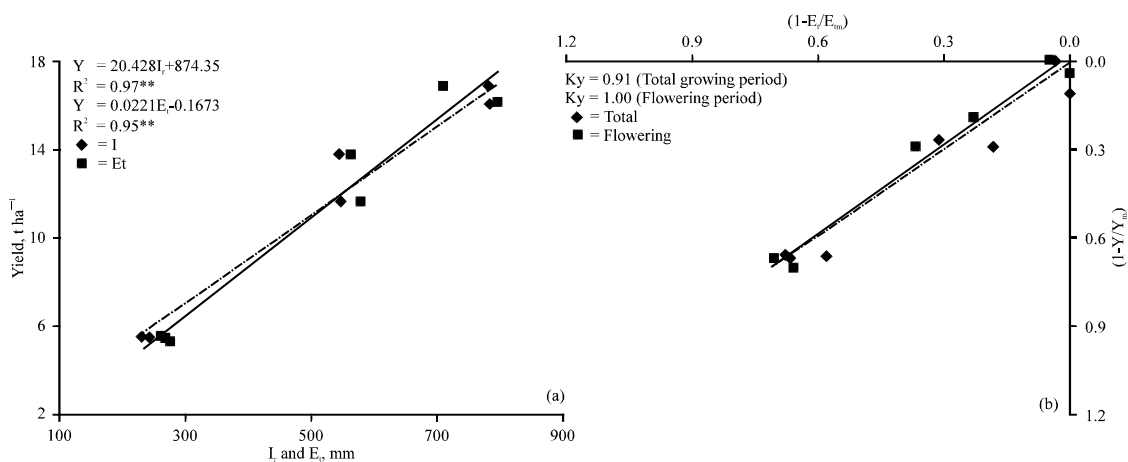


Fig. 1: Correlation among yield, I_r and E_t (a)-The relationships between relative yield decrease and relative evapotranspiration deficit for the total growing period (b)

Some fruit and plant growth traits: Some fruit and plant growth traits of irrigation treatments are presented in Table 2. Correlation equations and coefficients (R) among mean fruit weight, fruit length, fruit diameter, fruit number, irrigation water, E_t and yield are presented in Table 5.

Fruit number (FN): There was an increase in fruit number by I_r and E_t. There were significant positive linear correlations (p<0.01) between fruit number and both I_r and E_t. Increase in fruit number was one of the most significant (p<0.01) factor affecting the yield (Table 4)). Moreover, fruit number was significantly (p<0.001) affected by K_{cp}. While K_{cp}3 treatments produced the highest fruit number, K_{cp}1 treatments produced the lowest fruit number (Table 3). Thus, frequently and much more watered treatments increased the fruit number; consequently, the

fruit yield. However, irrigation intervals had no significant effect on fruit number.

Herrera *et al.* [20] find the similar results and indicated that irrigation intervals did not effect the fruit number. Chartzoulakis and Drosos [21] determined that both the fruit number per plant and fruit size were affected by the amount of water applied. Water shortage just prior and during early flowering period reduces the number of fruit. The effect of water deficit on yield during this period is greater under conditions of high temperature and low humidity [19].

Fruit Length (FL): Fruit length of the treatments had positively correlated with irrigation water, E_t and the fruit yield. There was a similar case as in fruit number. Increase in FL increased fruit yield (R²: 0.91**) more than increase

in fruit diameter. Wierenga [18] determined the fruit length values ranges between 11.8 to 14.1 cm and he stated that lack of water also reduced the length and the weight of the green pepper fruit. In our study similar results were obtained.

Fruit Diameter (FD): Fruit enlarged mostly with the increasing amount of irrigation water and E_i . There were significant correlations between FD and yield and between FD and both irrigation water and E_i ($p < 0.01$). Increase in fruit diameter was also one of the most significant ($p < 0.01$) factor affecting the yield.

Mean Fruit Weight (MFW): There were significant positive correlations ($p < 0.01$) between mean fruit weight and irrigation water, E_i or the fruit yield. Infrequently watered treatments had higher mean fruit weight than frequently watered ones. Moreover, in both irrigation intervals, treatments applied with the most amount of water had the highest mean fruit weight.

Plant Height (PH): Plant heights of treatments at the last harvest are showed in Table 2. The more irrigation water was applied, the higher the plant height was obtained. There were significant positive linear correlations ($p < 0.01$) between PH and irrigation water, E_i , the fruit yield, or the FN. Consequently, increase in PH increased the fruit number; therefore, the fruit yield. The plant height became the most important vegetative parameters affecting the fruit yield.

Plant Coverage (PC): Plant coverage increased by irrigation water and E_i (Fig. 2). Because if the environmental condition is favorable, the green pepper continues to grow and increase its canopy the growth period. Significant positive linear correlations ($p < 0.01$) were observed between PC and irrigation water, E_i , the fruit yield, or the FN. Increase in plant coverage increased the FN; therefore, the fruit yield. Enlargement in PC, an indicator of better plant growth, resulted in the enhancement of the plant photosynthetic area. Fruit yield increased in respect to performed photosynthesis. As plant develops, PC increases; therefore, E_i and photosynthesis get larger because transpiration increases [22].

The number of plant lateral branches (LBN): There were significant positive correlations ($p < 0.01$) between the number of lateral branches and irrigation water, E_i , the fruit yield the FN or plant coverage. The more LBN was, the

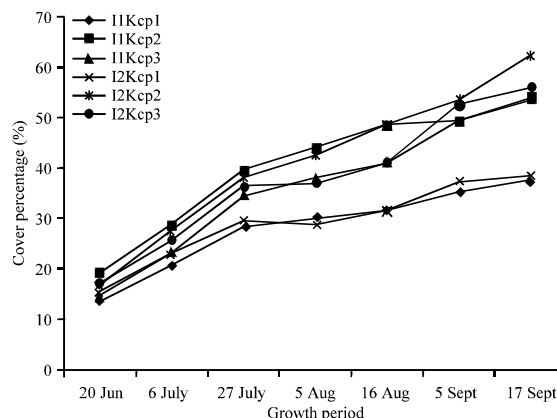


Fig. 2: Plant coverage in time

larger plant coverage was and the more FN was, therefore, the more the fruit yield was.

Plant Stem Diameter (PSD): Plant stem diameters of treatments at the soil surface level were measured in the last harvest. Significant positive linear correlations ($p < 0.01$) were observed between PSD and irrigation water, E_i , the fruit yield, LBN, or the FN. The larger PSD was, the more irrigation water, E_i and lateral branches, therefore, the fruit number and fruit yield were. Consequently, the stem diameter and lateral branches were among the most important vegetative traits increasing the fruit yield.

Soil water content before and after the irrigations: Soil water contents in the treatments measured at 90 cm depth of soil profile before and after the irrigations are shown in Fig. 3. While soil content was close to wilting point (110 mm) before irrigation, it tended to reach the field capacity (205 mm) after irrigations. I2 treatments were closer to wilting point before irrigation than I1 treatments. On the other hand, I2 treatments were closer to field capacity after irrigation than I1 treatments because water amount in I2 treatments per irrigation was more than the other. As stated in Meiri *et al.* [2], plants took more water from soil in infrequently irrigated treatments.

In general, soil content before and after irrigations was gradually decreased towards the end of the experiment. This might be due the fact that irrigation could not compensate plant water consumption and some of the previously stored water at soil profile was used up towards the end of the season. Because much more water was applied with increasing K_{cp} coefficients, the soil water content of treatments with high K_{cp} values were higher before and after irrigations than others. On the other hand, although the same amount of water was applied to

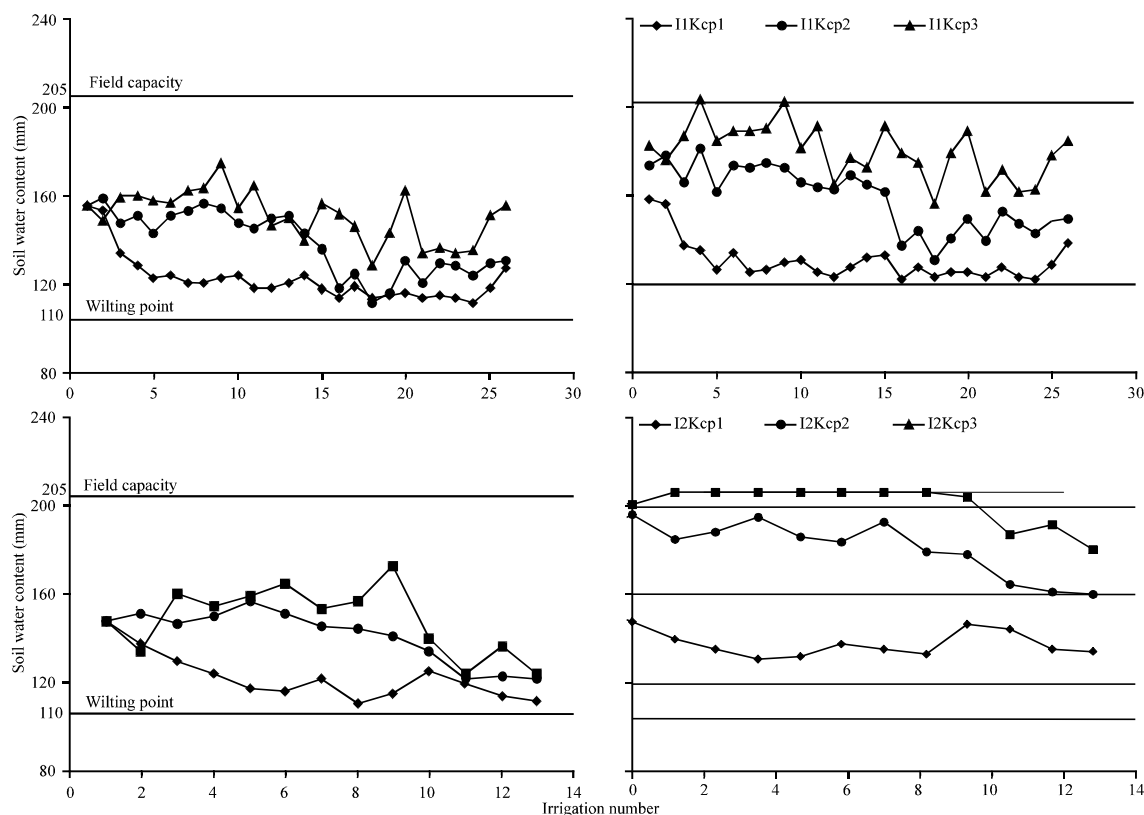


Fig. 3: Soil water contents measured at the initiation and after irrigations of I1 and I2 treatments

the both irrigation intervals, I2 treatments had a little bit more fruit yield than I1 treatments because I2 treatments were much closer to field capacity.

For optimum yield levels, the soil water depletion in most climates should not exceed 30 to 40 percent of the total available soil water. Light irrigation applications are required due to the low depletion level. Irrigation frequencies of 4 to 7 days are common [3]. Wierenga and Saddig [23] observed significant decreases in green pepper fruit yield as the water amount decreased in the soil. Moreover, they stated that it was necessary to irrigate green pepper plants before they used up more than 25% of the available water in the soil. Saddig [23] also determined a significant increase in crop water stress index for green peppers when more than 25% of the available soil water was taken up. In our study we had the similar results because we obtained more green pepper fruit yields from the treatments having more water in the soil before the irrigations. I2K_{cp}3 treatment where the highest yield obtained under 50±5 mm evaporation (about 4-5 day interval) and K_{cp}3, showed an agreement with above findings.

Water use efficiencies: Even though maximum fruit yields were obtained from K_{cp}3 treatments, irrigation water use efficiencies (IWUE) of these treatments were the lowest (Table 2). Although the total yield increased as irrigation water increased, the low yield amount per unit of irrigation water in K_{cp}3 treatments did not allow to get the highest economical yield from them. Costa and Gianquinto [24] informed that in most cases, WUE decreased with increasing water consumption, which was similar to our results. The highest IWUE values in frequently and infrequently watered treatments were obtained from the I1K_{cp}1 treatment (2.4 kg mG³) and I2K_{cp}2 (2.5 kg mG³) respectively. Treatments irrigated with higher amount of water had generally lower IWUE values. However, the irrigation frequency did not have any significant effect on IWUE. As it stated in Kanber *et al.* [25], treatments with low irrigation water amount but high fruit yield resulted in the highest IWUE values. Goldberg *et al.* [26] stated that irrigation time was more effective than total amount of irrigation water; when plants irrigated with limited amount of water in early growth stage, they grew better and their photosynthetic efficiency increased.

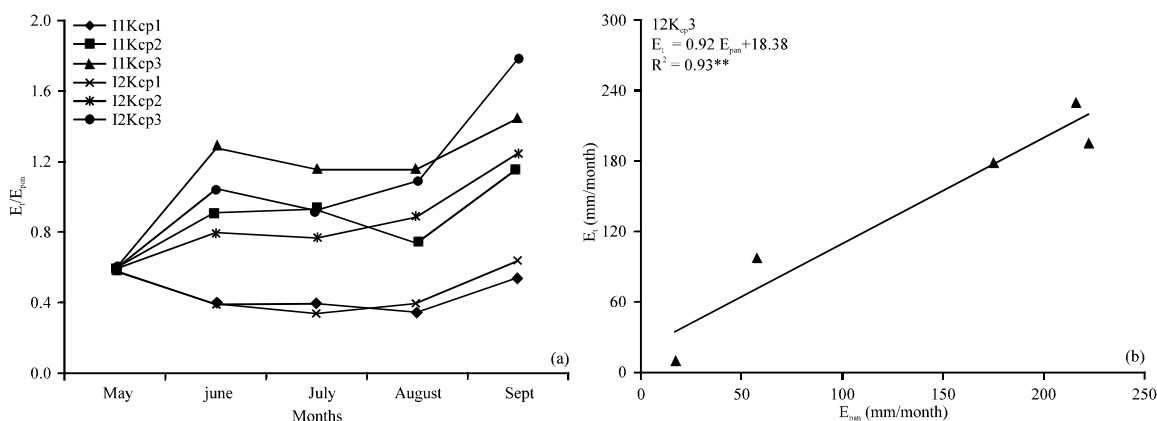


Fig. 4: Variation in E_t/E_{pan} ratio in growing period (a) and correlation between E_t and E_{pan} (b)

WUE values varied from 2.0 to 2.4 kg mG^3 and the highest values were determined in I2 treatments. Furthermore, because E_t increased with irrigation water, WUE values were close to IWUE values. It was reported that the WUE for harvested yield for fresh green pepper containing about 90 percent moisture varied between 1.5 and 3.0 kg mG^3 [27].

Irrigation compensations (I_{rc}) in both irrigation intervals were generally higher in treatments irrigated with high amount of water than those irrigated with low amount of water. I_{rc} values of I1 treatments were lower than those of I2 treatments. This was because plants in frequently watered treatments used much water and found water much more easily without encountering to water stress than those infrequently watered ones. Moreover, frequently irrigated treatments easily lost much more water by radiation due to the fact that the depth of the water applied once was lower than infrequently irrigated ones. Therefore, the soil water contents of I1 treatments before irrigation were much closer to wilting point than those of I2 treatments and plants in I1 treatments used more water than applied irrigation water. Consequently, in the areas where irrigation water is limited, I2K_{cp}2 treatment has to be taken into consideration in order to get the maximum yield per applied water amount because low WUE decreases productivity and increases crop production cost [28].

E_t/E_{pan} ratio: There was a significant positive linear correlation ($p < 0.01$) between E_t and E_{pan} (Fig. 4). This is in line with other studies [9, 28] showing a close relation between E_t and E_{pan} . Therefore, using pan evaporation in order to schedule the irrigations was a right and proper decision taken in this study.

E_t/E_{pan} curves of the same kind treatments were similar and they changed seasonally in the range from 0.34 to 1.76. The least watered treatments had the smallest E_t/E_{pan} ratio. E_t/E_{pan} rate of the all treatments inclined to increase until the last harvest. This is because of continuous inflorescence, fruit setting and fruit harvesting of green pepper plants until the last days of the long production season [29]. Moreover, continuous vegetative growth and enlargement in plant coverage also increased the E_t/E_{pan} ratio. At the end of the growing period, plants had larger canopies with many flowers on them and could not produce marketable acceptable fruit because of lower weather temperature after September; therefore, the production period was terminated. Because, a significant linear correlation ($p < 0.01$) was determined between E_t and plant coverage (Table 4). Wierenga [18] informed that there was increased E_t/E_{pan} ratios with increasing of leaf area index. Doorenbos and Kasam [15] stated that in annual plants, there were an increase in E_t/E_{pan} ratio in the middle of growing period, then this increase stabilized and E_t/E_{pan} ratio decreased at the end of the season. Furthermore, Goldberg *et al.* [26] informed that there was a positive linear correlation between E_t/E_{pan} ratio and plant canopy until plant canopy covered 80% of soil in plant rows. Our results were also in an agreement with this statement.

CONCLUSIONS

In this study, the highest green pepper yield (16.85 t haG^1) obtained from I2K_{cp}3 treatment which irrigated at 50 ± 5 mm evaporation interval with the highest amount of water. K_{cp} significantly affected the yield ($p < 0.001$); however, irrigation interval and IxK_{cp} interaction

had no significant effect on it. Yield response factor (K_y) was determined as 0.91. Fruit number was also significantly affected by K_{cp} . The highest yield per applied irrigation water was obtained from the I2 K_{cp} 2 treatment. Treatments irrigated with higher water amount had generally low IWUE and WUE values than others. I_{rc} of the treatments improved with the increasing amount of applied water. E/E_{pan} ratio among the treatments ranged from 0.34 to 1.76. There were also significant linear positive relationships ($p < 0.01$) among I_r , E_r , the plant growth and fruit traits. Increase in FN affected by LBN and SD improved the fruit yield. The highest earliest yield was obtained from K_{cp} 3 treatment which the most watered treatments.

In conclusion, although there was no significant effect of irrigation intervals, 50 ± 5 mm evaporation intervals with 1.10 of K_{cp} for peppers grown in field and climate conditions in Van or similar conditions can be recommended to obtain higher yield and to save time and labor. Furthermore, the equation ($E_r = 0.92 E_{pan} + 18.38$) determined for the K_{cp} 3 treatment can be applied in irrigation scheduling in pepper. Moreover, in the areas where the irrigation water is scarce, it will be more suitable to choose the I2 K_{cp} 2 treatment in order to get higher yield per applied irrigation water.

REFERENCES

- Campbell, O.S. and N.C. Turner, 1990. Plant-soil-water relationships. Management of farm irrigation systems (Hoffman G.J., T.A. Howell and K.H. Solomon, Eds.), The ASAE., 2950 Niles Road St. Joseph, MI., 15: 49085-9659.
- Meiri, A., H. Frenkel and A. Mantell, 1992. Cotton response to water and salinity under sprinkler and drip irrigation. Agron. J. Madison, Wis.: ASA, 84: 44-50.
- Anon, 2004, Faostat, 2003. <http://faostat.fao.org/faostat/form>.
- Garton, R.W. and J. Bodnar, 1991. Pepper production. Agriculture and Rural Division. Original factsheet, pp: 257.
- Berke, T.G., L.L. BLACK, S.K. Green, R.A. Morris, N.S. Talekar and J.F. Wang, 1999. Suggested cultural practices for chili pepper. International cooperators' guide, AVRDC pub., pp: 00-483.
- Elliadès, G., 1988. Irrigation of greenhouse grown cucumbers. J. Hort. Sci., 63: 235-239.
- Ertek, A., S. Sensoy, C. Kücükymuk and Ş. Gedik, 2004. Irrigation frequency and amount affect yield component of Summer Squash (*Cucurbita pepo* L.). Agric. Water Manag., 67: 63-76.
- Doorenbos, J. and W.O. Pruitt, 1975. Guidelines for predicting crop water requirements. Rome, FAO irrig. drain. paper No: 24.
- Kanber, R., 1984. Irrigation of peanut grown as primary and secondary crop in Cukurova by using free pan evaporation coefficient. (In Turkish). Bölge Toprak Su Arđ. Enst. Pub., Tarsus, 114: 93.
- James, L.G., 1988. Principles of farm irrigation system design. John Wiley and Sons. Inc., Newyork, pp: 543.
- Kanber, R., A. Yazar, S. Önder and H. Köksal, 1993. Irrigation response of pistachio (*Pistacia vera* L.), Irrig. Sci., 14: 1-14.
- Howell, T.A., R.H. Cuenca and K.H. Solomon, 1990. Crop yield response. Management of farm irrigation systems. (Hoffman, et al.). ASAE, pp: 312.
- Kanber, R., A. Yazar, H. Köksal and V. O-uzer, 1992. Evapotranspiration of grapefruit in the eastern mediterranean region of Turkey. Sci. Hortic., 52: 53-62.
- Stewart, J.I., R.E. Danielson, R.J. Hanks, E.B. Jackson et al., 1977. Optimizing crop production through control of water and salinity levels in the soil. Utah Water Res. Lab. Pub. No: PRWG 151-1, Logan, 191.
- Doorenbos, J. and A.H. Kassam, 1979. Yield response to water irrigation. Rome, FAO Irrig. drain. paper No: 33.
- Steel, R.G.D. and J.H. Torrie, 1980. Principles and procedures of statistics, 2nd Edn. McGraw Hill, Newyork.
- Beese, F., R. Horton and P.J. Wierenga, 1982. Physiological response of chili pepper to trickle irrigation. Agron. J., 74: 551-555.
- Wierenga, P.J., 1983. Yield and quality of trickle irrigated chile. Depart. of Crop and Soil Sci., New Mexico State Univ. Las Cruces, NM 88003. Agric. Exp. Station, Bulletin 703.
- Sagardoy, J.A., G.O. Bottrall and G.O. Uittenbogaard, 1986. Organization, operation and maintenance of irrigation schemes, FAO land and water development division, FAO irrigation and drainage paper 40.
- Herrera, A.L., A.D. Martinez and G.B. Lozano, 2002. Pepper production as influenced by irrigation frequency and plastic mulch. Proceeding of the 16th International Pepper Conference, Tampico, Tamaulipas, Mexico.
- Chartzoulakis, K. and N. DROSOS, 1997. Water requirements of greenhouse grown pepper under drip irrigation. Acta hort. (ISHS), 449: 175-180.
- Evsahibio-İu, A.N., 1989. Prediction of water consumption in pepper by remote sensation. (In Turkish). A.Ü. Ziraat Fakültesi Y2İİ2-2, Ankara, Vol: 40.

23. Saddig, M.H., 1983. Soil water status and water use of trickle irrigated chili pepper. Unpubl. Ph.D. Thesis. New Mexico State Univ., pp: 250.
24. Costa, L.D. and G. Gianquinto, 2002. Water stress and watertable depth influence yield, water use efficiency and nitrogen recovery in bell pepper: Lysimeter studies. *Aust. J. Agric. Res.*, 53: 201-210.
25. Kanber, R., O. Tekinel and N. Baytorun *et al.*, 1991. Estimation of the most suitable irrigation frequencies and quantities in cotton in Harran Plain by using free pan evaporation coefficient. (In Turkish) T.C. Bařbakanlık GAP Kalkınma İdaresi Bařkanlık GAP Pub. No: 44, Adana, pp: 15-25.
26. Goldberg, D., B. Gornat and D. Rimon, 1976. Drip irrigation-principles design and agricultural practices. *Drip Irr. Sci. Pub.*, Israel, pp: 295.
27. Anon, 2004, FAO, 2004. Crop water management. Pepper. AGLW water management group www.fao.org/ag/agl/aglw/cropwater/pepper.htm.
28. Bravo, L., G. Angel, L.Y. Jose and C. Chan, 1987. Relaciones aguasuelo-planta-atmosfera del maiz de riego en zonas semiaridas. I. rendimiento de grano. *TERRA*, 5: 132-139.
29. Çevik, B., N. Baytorun, Ç. Tanrıverdi, K. Abak and N. Sarı, 1996. Effect of different irrigation treatments on the yield and quality of eggplants grown in greenhouses. *TUBITAK. Turk. J. Agric. For.*, Ankara, 20: 175-181.

Wood Properties and Selection for Rotation Length in Caribbean Pine (*Pinus caribaea* Morelet) Grown in Afaka, Nigeria

Oluwadare A. Oluwafemi

Department of Forest Resources Management, University of Ibadan, Ibadan, Nigeria

Abstract: Plantations of Caribbean pine in Nigeria were established to provide the much needed local supply of long fibre pulp for the paper mills. Information on the biological characteristics of the wood is needed towards fibre utilization and selection of rotation length. The materials investigated in this study were woods from five age series –5, 7, 15, 20 and 25 years. These materials were characterized in terms of basic density, tracheid dimensions, kraft pulp and two growth parameters. Basic density increased with age as well as tracheid length and cell wall thickness. Ages 5 and 7 showed greater variability in these properties. Screened pulp yields increased with age with age 15 having the lowest screened yield permanganate ratio. Mean annual increment for tree height and diameter was highest at age 15. Findings showed that all the materials are suitable for papermaking with age 15 as the estimated rotation length for Caribbean pine, grown in Afaka, Nigeria.

Key words: Wood quality % tracheid dimensions % growth indices % *Pinus caribaea* (Morelet)

INTRODUCTION

The demand for wood and wood products is tremendously increasing worldwide. In Nigeria, the consumption of paper and paper products is daily increasing due to increasing awareness of computer technology and advancement in education. Many plantations of both exotic and indigenous species have been established to meet the quest for the required pulp need. Both short and long fibres are required to furnish good grade paper. The quality of paper to a certain extent depends on the quality of its fibres. In Nigeria, meeting the required tonnage of long fibre requirement is a problem moreover, that no single hardwood species have been found suitable to provide the much needed long fibre pulp. Plantation establishment of exotic pine species began as far back as 1960's. Among them, Caribbean pine proved most promising [1, 2]. The rotation length of many pines may be as long as 40 years. Due to competitive demand to which land is put to a shorter year may be preferred. For instance King [3] stated that pine pulpwood rotation length in the temperate region may be between 30-40 years and could be less in the tropics.

Rotation length is an important tool for controlling tree size [4] however; rotation length also markedly influences yield, product quality, profitability and regeneration methods. Rotation lengths are generally

determined based on management objectives along with biological characteristics of the commercial trees [5]. Many properties of wood depend on the age of the tree [6], when the wood was formed and the environment of the tree. These combined together with the genetic make up of the tree to produce the best wood for specific end-use. Since different paper and paper board products require different raw material characteristics [7], one cannot say that any one kind of raw fibre is desirable or undesirable without specifying the product. It is pertinent to specify the end-use product for which a tree crop is grown for before the selection of the final rotation length. All pine species grown in Nigeria are to provide long fibre pulp. It is therefore necessary to investigate the wood properties and the age at which these properties showed acceptable range value in meeting the objective of establishment.

This study investigates selected wood properties of Caribbean pine in combination with extrinsic growth parameters to determine appropriate rotation length.

MATERIALS AND METHODS

Samples of wood used in this study were obtained from five age series of *P. caribaea* grown in guinea savanna at Afaka, Kaduna Nigeria. The Afaka Forest Reserve is situated west of Kaduna on latitude 10°7'N and

longitude 7° 17'E on 600 m above sea level. Mean annual rainfall is about 1300 mm with daily minimum and maximum temperatures of 18°C and 24°C respectively. The trees selected were 5, 7, 15, 20 and 25 years old respectively. In each age group three trees were harvested with their total tree height and diameter at breast height measured. Discs of 5 cm in the thickness were obtained at breast height. Additional bolts of 20 cm for pulping materials were obtained at base, middle and top of trees sampled in 15, 20 and 25 age series while the entire logs from 5 and 7 age series was used. Based on the experimental design used, each age group was considered a treatment and each tree a replicate meaning five treatments with three replicate with a total sample of 15 trees.

Wood characterization: Basic density of the wood was based on the oven-dry weight and green volume of samples obtained from the disc.

Tracheids dimensions were measured based on inter-ring wood samples. The Splints obtained from each ring were macerated in equal volume (1:1) of 10% acetic acid and 30% hydrogen peroxide after the method of Franklin [8]. The bleached and soft splints were washed thoroughly in water and shaken in aqueous ethanol solution to free the tracheids. Two slides were prepared for each ring sample and 20 tracheids measured in swollen condition using Rleichart microscope for length (mm) L, diameter (µm) D, lumen width (µm) d and cell wall thickness (µm) w. From these, the derived morphological characteristics viz: felting power (FP), coefficient of flexibility (f) and wall fraction (WF) were determined. The obtained averages for each sample were used for statistical analysis.

Kraft pulping: A 25 litre rotatry laboratory digester was used for kraft pulping. The sampled materials were manually chipped and the following cooking parameters used for all samples:

C oven-dry weight of chips	= 2.0kg
C active alkali (% as Na ₂ O)	= 20
C sulphidity (%)	= 25
C maximum temperature (°C) (minutes)	= 170±2
C Time to maximum temperature	= 60 minutes
C Holding temperature (°C)	= 130
C Holding time (minutes)	= 30
C Time at maximum temperature (minutes)	= 180
C Liquor ratio	= 6:1

After cooking, pulps were washed on a stainless steel sieve with a mesh size 120 µ and later screened on 1-mm steel sieve. The clean pulp obtained was left in cold water with few drops of formalin before further analysis. The following parameters were determined:

- C total yield – ratio of oven-dry weight of pulp + rejects and oven dry weight of chips charged
- C screened yield – ratio between oven dry weight of screened pulp and oven dry weight of chips charged.
- C rejects – ratio between oven dry weight of total yield – oven dried weight of screened yield and oven dry weight of screened yield and oven dry weight of chips charged.
- C Permanganate number: This shows the degree of delignification of pulp or the amount of residual lignin in the pulp. This was carried out using TAPPI T-236 cm 85 as modified in the laboratory manual of Iwopin pulp and paper company test manual. Permanganate number is the volume (ml) of 0.1NKMnO₄ consumed by 2.0 gramme of oven-dry weight of pulp.

Estimation of rotation length: Rrotation length was first of all determined by identifying the end-use requirement of the pine plantations. The value of this objective was then calculated at each year of the stand's life (in this study-5-age series) using the extrinsic growth parameters (total height and DBH).

Mean annual increment (MAI) was used to estimate the rotation length [9] and vis-à-vis the various biological properties measured [10].

For total height, $MAI = \frac{TH}{age \text{ of stand}} \text{ (m/year)}$ and Diameter, $MAI = \frac{DBH}{age \text{ of stand}} \text{ (cm/year)}$

MAI was calculated for all stand ages and the age where it is greatest is chosen as the rotation age [11].

RESULTS

Basic wood density and Tracheid morphology: The mean wood basic density (WBD) and tracheid dimensions are shown in Tables 1 and 2. In Table 1, the least value of 407 kg/m³ was obtained for tree age 5 while the highest value of 497 kg/m³ was obtained for tree age 20. It is interesting to note that within-age class variation decreases with age as depicted by their coefficient of variation. It was highest in age class 5 and lowest in age class 25. These values are consistent with findings of

Table 1: Basic density of the wood

Material	Range	Basic wood density	
		kg/m ³ Average	CV
5-year-old	336-449	407a	15.1
7-year-old	369-451	408a	10.1
15-year-old	425-484	459b	6.6
20-year-old	465-529	497d	6.4
25-year-old	464-519	488c	5.8 ^a

values with the same letter are statistically different (DMRT at 0.05 significance level)

Table 2: Tracheid dimension and derived values

Material	L	D	d	w	Fp	Su (%)	WF (%)
5-year-old	2.34	59.64	47.62	6.01	39	79	20
7-year-old	2.44	54.22	40.78	6.62	45	76	24
15-year-old	2.64	59.14	46.50	6.47	44	78	22
20-year-old	3.23	58.32	42.68	7.82	56	73	27
25-year-old	4.23	62.08	43.07	9.50	68	69	31

L= tracheid length; D = diameter; d = lumen width; w = cell wall thickness; Fp = felting power (L/D); Su = suppleness/flexibility (d/D) x 100; WF= wall fraction (^{2w}/D) x 100

[12-15] on the same species from Jamaica, Fiji Island, Cuba and on maritime pine from France.

In Table 2, tracheid length increased with increasing age from 2.34 mm in age class 5 to 4.23 mm in age class 25. Variability in this trait was highest in age class 7 (10.7%) and least in age class 25 (4.0%). With exception of cell wall thickness, other tracheid parameters interspersely increased with age. Age class 15 seems a transition age between the juvenile phase and mature phase. It is only in this trait that within-class variation generally decrease with age from (0.8% in age class 5 to 1.4% in age class 25. Reports here is similar to [15, 16] on southern pines and 8-year-old trees from Brazil.

Pulp yield of kraft pulp: The performance of fibrous raw materials during pulping process is an indication of its quality for papermaking. Yields of pulps varied between age classes. The juvenile age classes (age class 5 and 7) had the highest total pulp yield of 55.1 and 52.2%, respectively. Similarly, the amount of rejects was more (8.3 and 6.1%). Age class 15 had the least screened yield of 45.5% and highest permanganate number (8.7%) but lowest screen yield permanganate number ratio of 5.2. The oldest age class (25 years) had the highest screened yield of 49.4%.

Table 3: Total yield, screened yield, rejects and permanganate number

Material	Total yield	Rejects	Screened yield:		
			yield	Permanganate number	permanganate number
5-year-old	55.10	8.26	46.62	8.5	5.5
7-year-old	52.18	6.09	46.09	7.6	6.1
15-year-old	47.58	2.04	45.54	8.7	5.2
20-year-old	49.26	1.99	47.27	5.7	8.3
25-year-old	50.42	1.06	49.36	8.5	5.8

Table 4: Mean tree height, diameter at breast height and their annual increment

Material	Tree height (m)	Diameter at breast height (cm)	MAI TH (m/year)	MAI DBH (cm/year)
5-year-old	4.4	2.4	0.88	0.49
7-year-old	5.7	3.3	0.81	0.47
15-year-old	17.7	26.1	1.18	1.74
20-year-old	22.0	26.5	1.10	1.33
25-year-old	22.0	26.1	0.88	1.04

Selection for rotation length: Table 4 shows the two growth parameters used (TH and DBH) with their derived MAI. The TH increased through the age classes however; circumferential increase in diameter was marginal from age 15 to 25. MAI for TH and DBH was highest at age 15 (1.18 m/yr and 1.74 cm/yr.) and thereafter decreased to 0.88 m/year and 1.04 cm/year at age 25. The need for short rotation length for pulpwood species has been emphasised [5, 14]. From Tables 1-4, the rotation length may be fixed at age class 15. At this age mean density, tracheid length, wall thickness suppleness and wall fraction were 459 kg/m³, 2.64 mm 6.47 μm, 78% and 22% respectively. These with the biological productivity of the trees (1.18 m/yr and 1.74 cm/yr combined with the management objective made age class 15 the suitable rotation length for *Pinus caribaea* at Afaka.

DISCUSSION

Basic density and Tracheid dimensions: The importance of basic density as a sole trait that is often measured in wood cannot be over-emphasised. It is a trait that gives indication of relative value of other wood properties such as strength properties, calorific value and pulp properties [14, 15, 17, 18]. Though basic density increased with age especially between the transition period of 15 years to mature period at 20 years due to increasing age as more mature wood is formed. However the higher rate of variability observed in the lower age series may be due to

juvenile nature of the wood. Generally, it is believed that juvenile wood varied considerably in their wood properties [6]. Whereas, mature wood is more stable hence lower variability, compare to samples from Tanzania, Cuba, Jamaica, Fiji Island, Brazil [12, 13, 15, 19] the differences observed may be due to the number of trees sampled and locations. Nevertheless, wood that is high in density in excess of 600 kg/m³ and above may not be suitable for papermaking [20]. Therefore the observed values are still within acceptable range for wood meant for pulp production.

It has been documented that tracheid properties of wood are of great importance in pulp and papermaking. The general increase in tracheid length as the tree matures with age is due to the aging of the cambium. The benefit of this is that better paper with higher paper strength will be produced provided there is no concomitant increase in other cell parameters especially cell wall thickness. Also the variation observed may be due to differences between juvenile wood (ages 5 and 7) and the mature wood (age 25) [15]. Variation in cell diameter and lumen width were not consistent, this may be due to the nature of individual tracheid that were selected for measurement. However, cell wall thickness increased with age with greater stability in the oldest trees (Table 1). This may not be unconnected with individual tracheid that constitutes the entire components of the cell wall substance.

The increase in derivatives of cell dimensions as observed for felting power is as a result of increasing cell length. This value however is lower than the values obtained by [11, 15, 21]. The disparity may be attributable to the longer cell length obtained in their studies compared to the shorter length in the present study. Nevertheless, suppleness and wall fraction were both similar to the values obtained by these authors. It is expected that the older trees may be difficult to refine compare to younger trees; however, in terms of strength the older trees will produce paper with greater strength properties especially in tear [6].

Pulp yield: From Table 3, screened yield of pulp was highest in the oldest tree. The increasing cell wall thickness may be responsible for this trend. This is further exemplified by higher wall fraction culminating in higher basic density. Also, the rejects in the older trees were generally low compared with younger trees with higher amount of rejects, low density and wall fraction which is typical characteristics of juvenile wood [15]. From this study, tracheid dimensions seem to have greater influence

on pulp yield and thus, it is expected that the final influence on paper properties will be advantageous. On the whole, the range of 45.5-49.4% for screen yields is still within acceptable range for most softwood tropical pine pulp yield [15, 21-23].

Selection of rotation length: There is a rapid and steady increase in demand for pulp products and an increasing shortage of wood supplies. Hence, short-rotation intensive culture plantations are being recommended [14]. In Tables 1-4, it may be suggested that age 15 be the most appropriate time to crop the trees for paper production. At this age, density was moderate while tracheid dimensions and its derivatives such as felting power, suppleness and wall fraction are still within acceptable standard for pines utilized for papermaking [15]. From age 15 upward, felting power and wall fraction increased while suppleness that determines to a greater extent inter-fibre bonding power was decreasing. Though, pulp yield was lowest, the value of ratio of screened yield to permanganate number showed that the yield is still within acceptable standard desirable for papermaking.

The extrinsic growth parameters (TH and DBH) evidently show that beyond age 15, the trees are growing at diminishing rate. This means that it may be economically unreasonable to keep the trees in the site bearing in mind the objective of establishment, the biological characteristics of the wood and production capacity of the forest sites [5]. To a fair approximation based on these findings, it is suggested that Caribbean pines grown in Afaka, a savanna zone in Nigeria should be cropped for pulp and paper production as the tree reaches age 15.

CONCLUSIONS

The five age series used in this study may be classified as juvenile wood-ages 5 and 7, transition wood-age 15 and mature wood-ages 20 and 25. Based on this classification ages 5 and 7 showed juvenile wood characteristics of low density, higher variability in wood properties, shorter cell length and thinner wall thickness with greater flexibility. In the other hand, mature wood was more stable in their properties with higher pulp yield, longer cell length but with thicker cell wall.

More importantly, the rotation length of Caribbean pine in the Afaka savanna zone should be 15 years. This is still subject to further studies as shorter length will be more preferable.

ACKNOWLEDGEMENTS

This project was self-sponsored with the assistance of Forestry Research Institute of Nigeria that gave the permission to fell the trees from their experimental plots at Afaka.

REFERENCES

1. Iyamabo, D.E. and M.A. Ogigirigi, 1976. Early growth patterns of pines in the Nigerian savanna. A paper presented at a symposia on savanna afforestation, Kaduna, Nigeria pp: 9-11.
2. Ogigirigi, M.A., 1994. Research extension and inter-institutional cooperation in Forestry in Nigeria. Invited paper at the 23rd Annual Conference of the forestry association of Nigeria, pp: 14.
3. King, F.S., 1975. It's time to make paper in the tropics. *Unasylva*, 27: 2-5.
4. Evans, J., 1992. Plantation forestry in the tropics. 2nd ed. Clarendon press, Oxford, pp: 403.
5. Baskent, E.Z., S. Kose and S. Keles, 2005. The forest management planning system of Turkey: constructive criticism towards the sustainable management of forest ecosystems. *International Forestry Review*, 7: 208-217.
6. Panshin, A.J. and C. de Zeeuw, 1980. Textbook of Wood Technology 4th ed. Publ. McGraw-Hill book Co. Inc. New York, pp: 772.
7. Food and Agriculture Organization 1990. Wood Structure, properties and utility. WT. 102-2, FAO working document, Rome 1990, pp: 35.
8. Franklin, G.L., 1945. Preparation of thin selection of synthetic resins and wood-resins composites and a new macerating method for wood. *Nature* 155: 3924-3951.
9. Husch, B., C.I. Miller and T.W. Beers, 1982. Forest Mensuration. 3rd edition John Wiley, New York, pp: 402.
10. Clutter, J.L., J.C. Fortson, L.V. Pienaar, G.H. Brister and R.L. Bailey, 1983. Timber Management: a quantitative approach. John Wiley and Sons, New York, pp: 333.
11. Leuschner, W.A., H.W. Wisdom and W.D. Klemperer, 1982. Multiple-use management of the forest: Introduction to forest Science ed. Young R.A. John wiley and Sons, pp: 261-288.
12. Palmer, E.R. and J.A. Gibbs, 1975. The pulping characteristics of two samples of *Pinus caribaea* from Cuba. TPI Report No. L. 41, pp: 25.
13. Palmer, E.R. and J.A. Gibbs, 1977, Pulping characteristics of *Pinus caribaea* from Fiji: Effect of rate of growth TPI. Report No. L. 46, pp: 16.
14. Pot, D., G., Chantre, P., Rozenberg, J.C., Rodrigues, G.L. Jones, H., Pereira, B. Hannrup, C. Cahalan and C. Plomion, 2002. Genetic control of pulp and timber properties in maritime pine (*Pinus pinaster* Ait.). *Ann. For. Sci.* 59: 563-575.
15. Gomes da Silva Junior, F., L.E.G., Barrichelo and C.E.B. Foelkel, 2004. Potential for multiple use of *Pinus caribaea* var. *hondurensis* wood with emphasis on pulp production. *J. For. Prod.*, 54: 42-49.
16. Koch, P., 1972. Utilization of the Southern Pines. Agric. Hands: USDA Forest Service, Washington, DC., pp: 1,663.
17. Stanger, T., B. Dvorak and G. Hodge, 2002. Variation and genetic control of basic wood density in *Pinus patula* grown in South Africa. <http://www.tappsa.co.za/archive/APPW 2002. 03/06/2006>.
18. Morling, T., 2002. Evaluation of annual ring width and ring density development following fertilization and thinning of Scots pine. *Ann. For. Sci.*, 59: 29-40.
19. Palmer, E.R., S. Ganguli and J.A. Gibbs, 1984. Pulping properties of *Pinus caribaea*, *P. elliotii* and *P. patula* growing in plantations in Kenya. TPI Report No. L. pp: 63-56.
20. Chittenden, A.E. and E.R. Palmer, 1990. Pulping characteristics of five low density wood species grown in Belize. *Tropical Science*, 30: 167-177.
21. Plumtre, R.A., 1984. *Pinus caribaea* vol. 2: Wood properties. Tropical Forestry Paper No. 17, CFI, Oxford, pp: 148.
22. Burley, J. and E.R. Palmer, 1979. Pulp and wood densitometric properties of *Pinus caribaea* from Fiji C.F.I. Occasional paper No. 6, pp: 66.
23. Kandeel, S.A.E., I.A. Kheraller and A.B. Elsayed, 1995. Chemical characteristics and pulp quality of SRIC *Pinus brutia* biomass in Egypt. IUFRO XX World Congress, 6-12 August, Finland, pp: 347.

Antagonistic Effect of Extracts of Some Nigerian Higher Fungi Against Selected Pathogenic Microorganisms

¹Jonathan Gbolagade, ²Loveth Kigigha and ²Elijah Ohimain

¹Department of Botany and Microbiology University of Ibadan, Ibadan, Oyo State, Nigeria

²Department of Biological Sciences, Niger Delta University, Wilberforce Island Bayelsa State, Nigeria

Abstract: *In-vitro* studies were carried out to investigate antagonistic effect of crude and purified extracts of some selected Nigerian higher fungi against selected pathogenic microorganisms. Purified and crude extracts of the tested higher fungi showed wide spectrum of antibacterial activity. The highest antibacterial inhibitory activity (24.0 mm) was recorded with the purified extract (PRE) of *Polyporus giganteus* against *E. coli*. The second widest zone of inhibition (22.0 mm) was recorded with the PRE of *Pleurotus florida* against *K. pneumoniae*. Except the extracts of *Pleurotus tuber-regium*, none of the tested macrofungi was able to inhibit the growth of *P. aeruginosa*. Generally, the antifungal activities of these higher fungi were low. Only *P. giganteus* and *T. robustus* inhibited the growth of *C. albicans* with values which are not statistically significant from each other ($p \# 0.05$). The minimum inhibitory concentration (MIC) of *M. jodocodo* against *E. coli* was 2.75 mg mL⁻¹ while that of *T. robustus* against *M. bourslerii* was 15.75 mg mL⁻¹. The implications of these findings were discussed.

Key words: Antagonistic % extracts % higher fungi % pathogenic microorganisms % *in-vitro*

INTRODUCTION

Nigeria is a country with many natural resources and vegetation which support the luxuriant growth of different types of naturally occurring higher fungi [1-4]. Edible macro fungi are usually collected from the wild because farms growing them are very few [2, 5].

In the southern part of Nigeria, people usually use fruitbodies and sclerotia of edible mushrooms as major food condiments which are served at their important family meals [6, 7]. Few higher fungi from Nigeria have also been reported to possess important medicinal ingredients among the traditional doctors [7-10].

Macro fungi that have been implicated of having curative effect against diseases such as high blood pressure, pneumonia, urinary tract infection, intestinal disorder by Nigerian herbalists include *Ganoderma lucidum*, *Fomes lignosus*, *Daldinia concentrica*, *Termitomyces species*, *Pleurotus species*, *Lycoperdon species*, *Polyporus species*, *Calvatia cynthiaformis* and *Psathyrella atroumbonata* [3, 8, 11].

Information on *in vitro* antimicrobial activities of these Nigerian higher fungi is very scanty or not

available in the literatures. Jonathan and Fasidi [7] reported that alcoholic extract of *Lycoperdon pusillum* and *L. giganteum* showed significant antimicrobial properties against some disease causing bacteria and fungi when compared with their respective water extracts. Likewise, Jonathan [3] reported that antibacterial potency of puffballs could be compared to some extent with the commonly used antibiotics. The objectives of this study is to evaluate the antimicrobial potentials of selected Nigerian higher fungi in view of the limited scientific information on their medicinal values.

MATERIALS AND METHODS

Higher Fungi: Eight [8] macro fungi including *Fomes lignosus* (Kl Bres), *Marasmius jodocodo* (Henn), *Pleurotus florida* (Mont) Singer, *Pleurotus tuber-regium* (Fries) Singer, *Psathyrella atroumbonata* (Pegler), *Polyporus giganteus* (Fries), *Termitomyces microcarpus* (Berk) and *Termitomyces robustus* (Beeli) were used for this study. The fruitbodies of these fungi were collected from Botanical Gardens, University of Ibadan, Ibadan Nigeria. They were identified using

the standard descriptions of Zoberi [1] and that of Alexopolous *et al.* [12].

Preparation of the Extracts: The sporophores of collected fungal samples were air dried under a shade for 5 days to avoid inactivation of the bioactive components by ultra violet radiation, then oven dried at 55°C for 48 hrs to a constant weight. The oven dried samples were milled to obtain fine powder. Eighty grammes (80.0 g) portion of the powdered samples were extracted with 320 ml of methyl-alcohol in a soxhlet apparatus for 6 h. The extracts were concentrated using a rotatory evaporator. The semi solid extract, thus obtained was further dried into powder form [3]. To obtain purified extracts, the solid crude extracts were mixed each with 1000 ml of sterile distilled water with stirring at 4°C overnight. The suspension, thus obtained were centrifuged to remove the insoluble matter; the aqueous supernatant was concentrated under reduced pressure to 200 ml. The concentrates were extracted with each 200 ml ethyl acetate and subsequently concentrated using rotatory evaporator to yield a light yellow material known as purified extract [13]. When required, both the crude and purified extracts were mixed with sterile distilled water to desired concentration.

Detection of antibacterial activity: The assay for antibacterial activities in the tested fungal sample was determined by agar well diffusion method described by Stoke and Ridgway [14]. Bacteria used were *Bacillus cereus*, *Escherichia coli*, *Klebseilla pneumoniae* *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staplylococcus aureus*. The pure culture of each bacterium was inoculated in peptone water for 18 hours, then seeded into nutrient agar plates (one organism per plate). Well (7 mm diameter) was made on each Petri dish using sterile cork borer. About 0.25 ml of the extract was introduced into bore agar wells using sterile dropping pipette. The plates were kept inside the refrigerator at 4°C for 12 hours to allow proper diffusion of the extracts into the medium. All the experiments were carried out in triplicates. Control experiments were also set up by adding 0.25 ml of sterilized distilled water into the well in place of the extract in three replicates. The plates were incubated at 37°C for 24 hours. The antibacterial activities of the extracts were expressed as the diameter of the inhibition zones (in mm) appeared on the inoculated plants

Detection of antifungal activities: The assay for antifungal potentials of these higher fungi extracts

was carried out using *A. niger*, *A. flavus*, *C. albicans*, *M. boulardii* and *T. concentrum* as test organisms seeded on to sterile plates of Sabouraud dextrose agar (SDA). Wells were made on the solid agar using 7 mm sterile cork borer. Twenty milligrammes (20.0 mg) of the extract was mixed with 5.0 g of the ointment base. Two grammes (1.5 g) of the mixture were introduced into the well on the agar plate. The control experiment was set up with the ointment base alone (without any extract). Each experiment was replicated three times. Each Petri-dish was inoculated with test fungus and incubated at 35°C for 7 days. The plates were observed for any zone of inhibition, which was measured in millimeters (mm).

Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration (MIC) was aimed at finding out the lowest concentration of the extract that will inhibit the growth of the tested microorganisms. In this experiment different concentrations (0.5 – 20.0 mg mL⁻¹) of the methyl alcohol extract were prepared by dissolving a known weight of the extract in a known volume of sterile distilled water. The mixture was tested against microorganisms using hole diffusion method. The test was first carried out by using high concentration of the extract (8.0 to 20.0 mg mL⁻¹) in a Completely Randomized Block Design. Those that were still effective at 8.0 mg mL⁻¹ were further diluted until no inhibitory zone was observed. The lowest concentration (dilution) produced was regarded as the minimum inhibitory concentration (MIC) for each extract [15]. Each experiment was carried out in triplicates. The sterile distilled water without any fungal extract served as the control.

Analysis of data: The data obtained were subjected to analysis of Variance (ANOVA) while the Text of significance were carried out using Duncan's multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The crude and purified extracts (CRE and PRE) of all the tested eight higher fungi used for this investigation possessed varying degrees of antibacterial properties against the tested bacteria (Table 1). Pure extract of *Polyporus giganteus* produced the widest zone of inhibition (24.0 mm) against *E. coli* followed by *P. atroumbonata* (18 mm) against the same bacteria (P#0.05). Pure extract of both *Fomes lignosus* and *T. microcarpus* produced inhibitory zones of 16.0 mm each against *E. coli*. On the other hand, *M. jodocodo*

Table 1: Antibacterial activities of crude and purified higher fungi extracts

Higher fungi	Test Bacteria					
	<i>B.cereus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. vulgaris</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
Zone of Inhibition (mm)						
F. lignosus (CRE)	15.0d	13.0gh	-	13.0fg	-	16.0bc
F. lignosus (PRE)	17.0b	16.0de	-	12.0g	-	17.0b
M. Jodocodo (CRE)	4.0j	-	10.0h	8.0i	-	13.0ef
M. jodocodo (PRE)	8.0i	-	13.0g	10.0h	-	17.0ab
P. florida (CRE)	-	13.0gh	20.0bc	-	-	16.0bc
P. florida (PRE)	-	13.0gh	22.0a	-	4.0b	18.0a
P. tuber-regium (CRE)	18.0a	8.0j	17.0de	16.0d	8.0a	12.0fg
P. tuber-regium (PRE)	18.0a	11.0i	19.0c	18.0bc	-	14.0de
P. atroumbonata (CRE)	12.0g	14.0fg	10	10	-	11.0gh
P. atroumbonata (PRE)	15.0d	18.0c	13.0g	14.0ef	-	15.0cd
P. giganteus (CRE)	13.0f	20.0b	13.0g	-	-	-
P. giganteus (PRE)	16.0c	24.0a	16.0ef	-	-	-
T. microcarpus (CRE)	-	13.0gh	-	17.0cd	-	16.0bc
T. microcarpus (PRE)	-	16.0de	-	20.0a	-	18.0a
T. robustus (CRE)	10.0h	12.0hi	13.0g	-	-	6.0i
T. robustus (PRE)	14.0e	15.0ef	15.0ef	-	-	10.0h
Control (Distilled water)	-	-	-	-	-	-

Key: CRE = Crude extract PRE = Purified extract

Values followed by the same letter(s) along each column are not significantly different by Duncan's multiple range test (DMRT) ($p \geq 0.05$)

Table 2: Antifungal activities of crude and purified higher fungi extracts

Higher fungi	Test Fungi				
	<i>A. niger</i>	<i>A. flavus</i>	<i>C. albicans</i>	<i>M. bouldardii</i>	<i>T. concentrum</i>
Zone of Inhibition (mm)					
F. lignosus (CRE)	-	-	-	-	-
F. lignosus (PRE)	-	-	-	-	-
M. jodocodo (CRE)	5.0e	7.0e	-	-	-
M. jodocodo (PRE)	9.0cd	8.0de	-	-	-
P. florida (CRE)	-	-	-	-	-
P. florida (PRE)	-	-	-	-	-
P. tuber-regium (CRE)	-	-	-	-	-
P. tuber-regium (PRE)	-	-	-	-	-
P. atroumbonata (CRE)	8.0d	10.0c	-	5.0c	-
P. atroumbonata (PRE)	10.0bc	12.0ab	-	8.0ab	-
P. giganteus (CRE)	10.0bc	12.0ab	9.0a	-	9
P. giganteus (PRE)	11.0ab	13.0a	10.0a	-	-
T. microcarpus (CRE)	-	-	-	-	-
T. mirocarpus (PRE)	-	-	-	-	-
T. robustus (CRE)	10.0bc	-	7.0a	5.0c	-
T. robustus (PRE)	12.0a	-	10.0a	9.0a	-
Distilled water (control)	-	-	-	-	-

Key: CRE = Crude extract PRE = Purified extract.

Values followed by the same letter(s) are not significantly different byDuncan's multiple range test (DMRT) ($p \geq 0.05$)

Table 3: Minimum inhibitory concentration for bacteria and fungi

Higher fungi	Test bacteria and fungi								
	<i>B. cereus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. vulgaris</i>	<i>S. aureus</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>C. albicans</i>	<i>M. bouldarii</i>
	MIC (mg mL ⁻¹)								
<i>F. lignosus</i>	5.50de	6.00bc	-	4.75c	4.50e	-	-	-	-
<i>M. jodocodo</i>	7.25a	2.75e	6.00b	8.75a	7.50a	13.75b	15.50a	-	-
<i>P. florida</i>	-	7.25a	7.75a	-	6.00b	-	-	-	-
<i>P. tuber-regium</i>	5.00e	-	6.25b	3.50d	-	-	-	-	-
<i>P. atroumbonata</i>	6.50b	5.00c	6.00b	4.25c	4.00e	14.00ab	15.25a	-	13.50b
<i>P. giganteus</i>	5.75cd	3.75d	3.25c	-	4.75de	10.50c	12.00c	13.25a	-
<i>T. microcarpus</i>	-	7.00a	-	6.00b	5.00cd	-	-	-	-
<i>T. robustus</i>	6.00bc	5.75	3.50c	-	6.25b	14.25a	14.00b	13.75a	15.75a

Values followed by the same letter(s) are not significantly different by Duncan's multiple range test (DMRT) (p#0.05)

possessed no antibacterial activities. The strong antibacterial properties possessed by *P. giganteus* and *P. atroumbonata* is not a surprise, because these two fungi are important part of medicinal ingredients which are used by the local Yoruba people in the south western Nigeria for the treatment of intestinal disorder and some other bacterial infections [3].

All the tested extracts (either pure or crude) except those of *P. giganteus*, inhibited the growth of *S. aureus*. The purified extract (PRE) of both *P. atroumbonata* and *T. microcarpus* had the best *in-vitro* antibacterial activities (18.0mm inhibition zone) against *S. aureus* (Table 1). It was interesting to note that *P. aeruginosa* which is resistant to both tetracycline and gentamycin [3, 16] was found to be sensitive to the methyl-alcohol extract of *P. tuber-regium*. The potent antibacterial activity exhibited by *P. tuber-regium* against most of the tested bacteria supported the earlier report of Oso [9, 11] that *P. tuber-regium* is a medicinal mushroom.

Klebsiella. pneumoniae was inhibited by all the extracts except *F. lignosus* and *T. microcarpus* (Table 1). This observation suggests that these fungi contained potential antibacterial agents against infection from this organism. Oso [8, 9] reported that *T. microcarpus* is a powerful medicinal ingredient for the treatment of gonorrhoea among the traditional doctors in the south-western Nigeria. This medicine which is administered orally is prepared by grinding a large quantity of *T. microcarpus* with the pulp of the fruit of *Cucurbita pepo* Linn.; the leaves of *Cassia alata* Linn and some other ingredients.

From Table 2, it was clearly revealed that the antifungal properties of the tested higher fungi were generally poor. Only four of the eight screened mushrooms exhibited weak antifungal properties

against at least two pathogenic fungi. Only crude extract of *P. giganteus* showed inhibitory effect against the dematophyte (*T. concentrum*). Likewise, *P. atroumbonata* and *T. robustus* inhibited the growth of *M. bouldarii*. This result was similar to that reported by Jonathan and Fasidi [10] for *D. elegans* and *C. occidentalis*. The extracts of *P. giganteus* and *T. robustus* weakly inhibited the growth of *C. albicans* while other tested mushrooms showed no antifungal properties against this fungus. Similar inhibitory effect against *C. albicans* was observed by Jonathan [3] for *C. occidentalis* and *D. concentrica*.

It was generally observed that purified extract (PRE) of the tested macrofungi exhibited more potent antimicrobial activities than crude extracts (CRE). (Tables 1 and 2). The values obtained for CRE and PRE for *M. jodocodo* against *B. cereus* were 4.0 and 8.0 mm respectively. Similar result was obtained for this mushroom against *A. niger* and *A. flavus* (Table 2). Eunjeon *et al* [17] and Kenji *et al* [18] reported similar observation with *Ganoderma lucidum* and *Hericium erinaceum* respectively. Tan and Moore [19] Irinoda *et al*. [20]; and Tochikura *et al* [21] separately observed that purified extracts of edible mushrooms are more effective against microorganisms than crude extracts.

Table 3 shows that the minimum inhibitory concentration (MIC) of the extracts ranged between 2.75 and 15.75 mg mL⁻¹. The lowest MIC (2.75 mg mL⁻¹) was found with the extract of *M. jodocodo* against *E. coli*. This was followed by *P. giganteus* extract against *K. pneumoniae*. *Pleurotus tuber-regium* and *T. robustus* had the MIC of 3.50 mg mL⁻¹ each against *P. vulgaris* and *K. pneumoniae* respectively. Danielli [22] suggested that the lower the MIC, the more sensitive and promising the extract. This implies that most of these

higher fungi offer potential therapeutic potency against some of the medically important bacteria. The MIC against fungi were generally high. This result confirms the observation made that the higher fungi studied possessed poor antifungal activities.

REFERENCES

1. Zoberi, M.H., 1972. Tropical Macrofungi. Macmillan Pres London, pp: 158.
2. Alofe, F.R., E.A. Odu and H.C. Iloh, 1998. Mushrooms, man and Nature. Edible wild mushrooms on decaying wood in Obafemi Awolowo University Campus. The Nigerian Fields 63: 3-18.
3. Jonathan, S.G., 2002. Vegetative growth requirements and antimicrobial activities of some higher fungi in Nigeria. Ph.D. thesis University of Ibadan. Ibadan, Nigeria.
4. Gbolagade, J.S., A, Ajayi, I. Oku and D. Wankasi, 2007. Nutritive value of common wild edible mushrooms from southern Nigeria. Global Journal of Biotechnology and Biochemistry (In Press).
5. Fasola, T.R., J.S. Gbolagade and J.O. Fasidi, 2007. Nutritional requirements of *Volvariella speciosa* (Fr Ex. Fr) Singer, a Nigerian Edible Mushroom. Food Chemistry, 100: 904-908
6. Fasidi, I.O. and U.U. Ekuere, 1993. Studies on *Pleurotus tuber-regium* (Fries) Singer: Cultivation, proximate compositions and mineral contents of the sclerotia. Food Chemistry, 48: 255-258.
7. Jonathan, S.G. and I.O. Fasidi, 2003. Antimicrobial activities of two Nigerian edible macrofungi *Lycoperdon pusillum* (Bat. Ex) and *Lycoperdon gigantum* (Pers). African Journal. of Biomedical Research, 6: 85-90.
8. Oso, B.A., 1977a. Mushrooms in Yoruba mythology and medicinal practices. Eco. Bot., 31: 367-371.
9. Oso, B.A., 1981. Fungi and mankind. University of Ibadan, Ibadan (Nigeria) Inaugural Lecture, pp: 40.
10. Jonathan, S.G. and I.O. Fasidi, 2005. Antimicrobial activities of some selected Nigerian Mushrooms. Afr. Jour. of Biomedical Research, 8: 83-87.
11. Oso, B.A., 1977b. *Pleurotus tuber-regium* from Nigeria. Mycologia, 67: 271-279.
12. Alexopolous, C.J., C.W. Mims and M. Blackwell, 1996. Introductory Mycology (4th Ed). New York John Wiley.
13. Hirasawa, M., M. Shoujii, T. Neta, K. Fukushima and K. Takada, 1999. Three kinds of antibacterial substances from *Lentinus edodesi*. (Berk) Sing. (Shitake, an edible mushroom). Int. Journ. of Antimicrobial Agents, 11: 151-157.
14. Stoke, J.E. and G.L. Ridgway, 1980. Clinical Bacteriology. Edward Arnold Ltd. London.
15. Pelazar, M.J., E.C.S. Chan and N.R. Krieg, 1993. Microbiology: Concepts and Application. MacGraw-Hill Inc. New York pp: 967.
16. Madigan, M.T., J.M. Martincio and J. Parker, 1997. Brock Biology of Microorganisms. (8th Ed.) Prentice Hall Int. Inc.
17. Eun-Jeon, P., R. Geonil, K. Jaebae and H.C. Dong, 1997. Antifibrotic Effects of Polysaccharides extracts from *Ganoderma lucidum*. Biol. Pharm. Bull., 20: 417-420.
18. Kenji, O., Atsushis, S. Ryoko, S. Hideki, Y. Satoshi, O. Eumihiro, I. Yukio, S. Takuo and Hirokazu, 1993. Antimicrobial Chlorinated orcinol derivations from mycelia of *Hericium erinaceum*. Phytochemistry, 34: 1445-1446.
19. Tan, Y.H. and O. Moore, 1994. High concentration of mannitol in shiitake mushroom: *Lentinula edodes*. Microbios, 79: 31-35.
20. Irinoda, K., N.K. Mashihi, G. Chihara, Y. Kaneko and T. Katori, 1992. Stimulation of microbicidal host defence mechanism against aerosol influenza virus infection by *Lentinan* Int. J. Immunopharmal, 14: 971-977.
21. Tochikura, T.S., H. Nakashima, Y. Ohashi and N. Yamamoto, 1988. Inhibition (*in-vitro*) of replication and the cytopathic effect of human immunodeficiency virus by an extract of culture medium of *Lentinus edodes* mycelia. Med. Microbiol. Imm., 177: 235-244.
22. Danielli, I., 1957. Antimicrobial activities of *Aframomium* species. Pharm. J., 16: 470-472.

Women Farmer and Their Educational Needs in Small Ruminant Production in the Northern Badia Region of Jordan

Laith M. Rousan

Department of Plant Production, Faculty of Agriculture, Extension and Transfer of Technology,
Jordan University of Science and Technology, Irbid 22110 - Jordan

Abstract: The primary purpose of this study was to describe selected demographic characteristics of rural women farmers in the Badia region of Northern Jordan, to assess their perceived agricultural educational needs and perceived barriers to extension participation. Data were collected from 260 rural women farmers. A reliable and valid survey questionnaire was developed and data was collected by using face-to-face interviews. For this study, four objectives were developed: (a) to describe rural women according to selected characteristics (age, marital status, level of education, farming experience and farm size), (b) to determine the perceived educational needs of rural women, (c) to determine perceived barriers to Extension participation by rural women, (d) to determine the relationship between selected demographic characteristics of rural women and their perceived educational needs and barriers to Extension participation. Perceived educational needs were assessed using the Borich (1980) needs assessment model. Findings revealed that rural women's highest educational needs were in livestock production, nutrition and resource management and marketing and outstanding barriers to Extension participation lack of information about Extension activities, Extension agents do not often organize training programs for rural woman, heavy loads of household task and time constraints, Results of the study can help Extension Departments related to the Ministry of Agriculture and the Badia Development Center in Jordan in placing its priorities on the items that were ranked high to meet the needs of rural women, attract a wider audience and lead to the success of Extension programs.

Key words: Rural women % women farmers' % educational needs % small ruminant % extension service

INTRODUCTION

Women play a major role in small ruminant production. The foremost tasks of women in small ruminant production are milking, cleaning barns, cutting and carrying grasses, grazing and mixing fodder. Women contribute a significant percentage of the labor to small ruminant production; however, it is not always recognized because men hold the structural authority. Despite women's significant role, educational and/or training programs about small ruminant production regarding women in rural areas are far from an acceptable level [1-6]. Rural women, play a significant role in many agricultural activities in many countries. Women activities include plant and animal production activities such as production of food for the household, planting and weeding, harvesting and post harvest activities, livestock care and

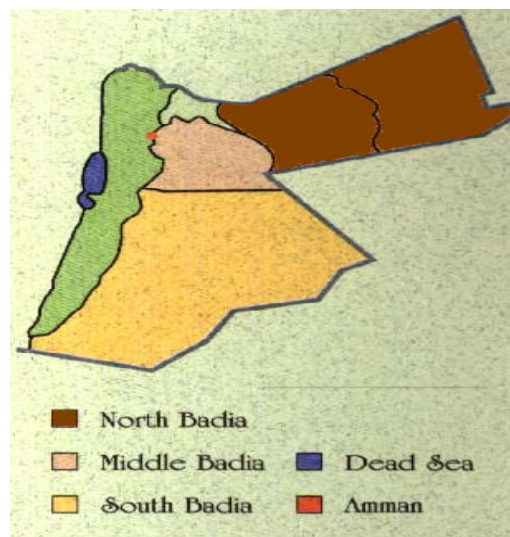
commercial farming. Specific tasks and activities are regarded in some societies as predominately female work. They are generally tedious and time consuming tasks and considered as household duties rather than work. Women time and mobility are constrained by their multiple domestic, reproductive and agricultural roles. Besides, there are more barriers that prevent women from improving their productivity than men.

Although women are the main actors in feeding the household, they often have little or no access to land, credit, education and technology, little attention has been paid to alleviate women's problems, particularly those in rural areas. Due to gender blindness that still prevails, agricultural policies, on the whole, do not address the needs of women farmers adequately [7]. Rural women have been suffering serious problems all over the world. The situation has been worse in the

developing countries generally, despite the existence of plans and policies for integrating rural women in to the development process. Rural women are also disadvantaged with regard to education and health: 30.3% of rural women are still illiterate, as compared to 17.8% of women living in urban settlements. Although their rate of participation in the basic education cycle does not differ, significantly, less rural women than urban women acquire secondary school and higher education. This is partly due to the general lack of secondary schools and colleges outside the main cities, but it is greatly exacerbated by the fact that those fewer available facilities cater first and foremost to male students [8].

Rural women have a much reduced access to agricultural extension services worldwide compared to men and technology is rarely designed specifically to address their gender-based needs. In Africa only 7 percent of all agricultural extension resources were allocated to women farmers and home economic extension received only 1 percent of the resources. In the Sudan, for example in the Gezira irrigation scheme out of 120,000 farmers targeted by the agricultural extension services only 11 percent were women. The main constraints limiting women's access to extension services were related to cultural restriction, domestic responsibilities, mobility limitations and even language barriers [7]. Furthermore gender disparity in extension programs has long been acknowledged. Women were excluded from the benefits of extension. Even though women have an enormous role in animal husbandry, most of the extension programs are designed to target men. The identification of gender roles in small ruminant production and management can help extension, veterinary and research institutions to develop appropriate educational programs and research. Women farmers' access to extension services will enhance small ruminant production and household food security. Extension educators are responsible for helping farmers to accurately identify their educational needs. This is an important step in planning, developing and implementing extension programs [9]. Programs are most often successful when they focus on clearly defined needs of the target group [10]. Therefore, the accuracy with which needs are identified for educational input is a crucial step toward meeting Extension's objectives.

Purpose and objectives: The arid lands or Badia is one of the concerns that have been studied during the twentieth century in Jordan. It covers a wide and significant part of Jordan (18%) of the total area which is



The Hashemite Kingdom of Jordan
The Location of Jordan Badia

Fig. 1: Location of Jordan (Map)

approximately 72,600 km². This region is subdivided into three geographical areas:

- C Northern Badia which comprises 35% of the Badia total area
- C Middle Badia which comprises 13% of the Badia total area.
- C Southern Badia which comprises 51% of the Badia total area.

Although the vegetation cover is not dense and surface water mostly absent, the potential pastureland covers large part of the Badia. About 61% of farm animals in the country are located in Badia and around 70% of Jordan's animal products are produced in it [11]. However; this study focused on the Northern Badia region where most of the farms in this area are classified as family farms and the main economic activity is small ruminant production. The questionnaire was implemented face-to-face interview by the author and a female trained team of data collectors. The survey was conducted between the middle of 2005 and the end of 2006.

The primary purpose of this study was to describe selected demographic characteristics of rural women in the Badia region of Northern Jordan, to assess their perceived agricultural educational needs and perceived barriers to extension participation. For this study, four objectives were developed:

- C To describe rural women in the Northern Badia, according to selected characteristics (age, marital status, level of education, farming experience and farm size).
- C To determine the perceived educational needs of rural women in the Badia region of Northern Jordan.
- C To determine perceived barriers to Extension participation by rural women in the Northern Badia region.
- C To determine the relationship between selected demographic characteristics of rural women and their perceived educational needs and barriers to Extension participation.

Methodology and procedures: The research design in this study was descriptive correlational survey. The target population for this study was rural women in the Badia region of Northern Jordan. A sample of 260 rural women was selected from ten randomly selected villages in the Badia region. The survey instrument was developed and tested for validity and reliability prior to implementation. Data were collected using face-to-face interviews. The survey instrument elicited three categories of information from the participants: (a) demographic data, (b) perceived barriers to Extension participation, (c) assessments of self perceived amount of “knowledge” for agricultural production and assessment of self perceived level of “importance” in agricultural production. The descriptors for “knowledge” and “Importance” scales were: “4” = “High knowledge”/“High Importance” ... “0” = “No knowledge”/“No Importance.” Perceived educational needs, the dependent variable, were assessed using Borich needs assessment model [12]:

$$\text{Equation 1: } \text{Cal Aen} = (\text{In} - \text{Kn}) (\text{Ig})$$

Where:

- Cal Aen = calculated educational need.
- In = importance of the item reported by the respondent.
- Kn = perceived knowledge of the item reported by the respondent.
- Ig = average importance of the item as rated by all the respondents.

Participants rated each educational need twice according to the four-point Likert type scale provided, first they rated it as to the amount of knowledge they currently possessed and secondly they rated it in terms of its importance in increasing agricultural production. Data

Table 1: Demographic characteristics of the respondents (N= 260)

Characteristics	M	SD
Age	39.17	9.83
Years of schooling	6.90	3.50
Household size	6.95	2.70

Table 2: Background Characteristics of the respondents (N=260)

Characteristics	f	%
Marital status		
Married	205	78.85
single	29	11.15
Widow	23	8.85
Divorced	3	1.15
Land ownership		
Wife	19	7.31
Husband	204	78.46
Jointly	11	4.23
Other	26	10.00
Ability to read and write		
Yes	206	79.23
No	54	20.77

were analyzed using the Statistical Package for Social Sciences (SPSS). Statistical analysis included descriptive, correlations and multiple regressions. Missing item values were handled by using mean substitution [13].

Findings:

Demographic characteristics: Characteristics of participants in this study are summarized in Tables 1 and 2. Table 1 presents the means and standard deviations for the demographic characteristics that were measured using ratio scales. The mean age of rural women in this study was 39 years. The youngest respondent was 18 years of age and the oldest was 72. A majority of the participants had completed 1 to 8 years of schooling and almost one- quarter never attended school. The average period of time spent in school was 6.90 years with the minimum being 0 and maximum being 16 years. A majority of households (61%) in this study had 6-10 members and 8% had more than 11 members.

Table 2 presents the frequency and percentages of the background characteristics of the participants that were measured using nominal scales. Data revealed that majority of rural women (79%) in this study were married, eleven percent was single and nine percent were widowed. In respect of land ownership, seventy eight percent owned by the husband, ten percent did not own land, seven percent was owned by the wife separately and four percent of the rural women jointly owned land with their husband. Most of the participants

Table 3: Rank order of the calculated educational needs

Statement	Rank	M	SD
Milk processing techniques	1	6.14	5.97
How to control livestock diseases	2	5.83	5.72
Marketing of their product	3	5.41	5.34
Plan and prepare balanced meals	4	5.12	5.23
How to determine when to sell	5	4.87	4.68
Animal feed blocks formulation and use	6	4.74	4.81
Profitable animals selection to keep	7	4.52	4.65
Use of crop residue as a fodder	8	4.41	4.47
How to access loans	9	4.39	4.72
Food preservation	10	4.36	4.88
Use of uterus synchronization sponges	11	4.35	4.96
Book keeping records	12	4.29	4.57

Table 4: Rank order of bottom 12 educational needs

Statements	Rank	M	SD
Water harvesting techniques	41	2.81	3.72
Adding or and injecting vitamin (AD3E)	42	2.75	3.62
Range land management	43	2.63	3.82
Select suitable harvesting methods	44	2.59	5.43
Correct fertilizer for crops	45	2.48	5.14
How to control weeds	46	2.39	5.01
Information on profitable crops to grow	47	2.27	4.10
Choosing high quality seeds	48	2.13	4.33
Identify weeds that affect crops	49	2.04	3.25
Selection of suitable crop varieties	50	2.01	3.11
Preparation of land for planting	51	1.97	3.49
How to plant crops	52	1.82	3.58

(79%) in this study could read and write and twenty one percent could not.

Agricultural educational needs: Using the Borich's model [12], a higher mean indicates a greater educational need. The ranks, means and standard deviations of 12 highest educational needs of rural women are provided in Tables 3. As shown in Table 3, the highest educational need was milk processing techniques, followed by controlling of livestock diseases. Four among the top 12 highest educational needs were related to nutrition and five are related to resource management and marketing of flocks (marketing, when to sell, profitable animals to keep, how to access loans, book keeping records).

Table 4 provides ranks, means and standard deviations of 12 lowest educational needs of rural women. As illustrated in Table 4, nine of twelve least important educational needs were related to crop production.

Perceived barriers to extension participation by participants: The third objective of the study was to determine the perceived barriers to Extension participation

Table 5: Rank order of perceived barriers to extension participation

Statements	Rank	Ma	SD
Lack of information about extension activities	1	2.92	1.45
Extension agents do not often organize training programs for rural woman	2	2.73	1.84
Heavy loads of household tasks\time constraint	3	2.58	1.32
Permission by husband	4	2.47	1.26
Extension training programs do not include woman's training needs	5	2.29	1.18
No access to credit	6	2.23	1.04
Lack of female extension agents	7	2.01	1.13
Social and cultural customs prevents rural woman from attending extension activities	8	1.95	1.19
Extension training sites are far from where most woman live	9	1.86	1.17
Woman's inability to read and write	10	1.83	1.12
Lack of child-care facilities	11	1.79	1.14
No land or access to land	12	1.68	1.08

Note ^aScale ranges from 1-4; 1 = Strongly Disagree; 2 = Disagree; 3 = Agree; 4 = Strongly Agree

by rural women. Table 5 provides ranks, means and standard deviations of the perceived barriers to Extension participation by rural women. Barriers to Extension participation scores ranged from a mean of 1.68 to a mean of 2.92. As illustrated in Table 5, the highest barriers were: 1) Lack of information about extension activities, 2) Extension agents do not often organize training programs for rural woman, 3) Heavy loads of household tasks\time constraint, 4) permission by husband and 5) Extension training programs do not include woman's training needs.

Demographic characteristics and agricultural educational needs:

The fourth objective of the study was to determine the relationship between selected demographic characteristics of rural women and 1) their perceived educational needs in the four areas and 2) their perceived barriers to Extension participation. Using Borich's model, an overall educational need score was computed for each of the four areas of domain. An overall mean score of each of 12 barriers was computed. These mean scores were treated as interval data. Correlations coefficients were calculated among the mean scores of the calculated needs, the barriers and the selected demographic characteristics.

Table 6 reports correlation coefficients among selected demographic characteristics and the four areas of perceived educational needs. A low association (0.11) existed among crop production and years in school. Negligible associations existed among livestock

Table 6: Correlation coefficients among selected demographic Characteristics of rural woman and the four areas of educational needs

Area of agricultural educational needs	Correlation coefficients (r)	
	Age	years of schooling
Livestock production	0.04	-0.02
Crop production	-0.08	0.11
Resource management and marketing	-0.06	0.04
Nutrition knowledge	0.05	-0.06

production and age, years in school and resource management and marketing. The correlation among nutrition knowledge and age was negligible. Resource management and marketing and crop production had negative association with age. Negative associations existed among years in school and livestock production and nutritional knowledge.

All the barriers were negatively associated with age except for one barrier (women’s inability to read and write) with a negligible association of .05. The following barriers had low associations with number of years in school: 1) no access to credit (0.12) and 2) Extension agents do not often organize training programs for rural women (0.14). Two of the barriers (lack of female extension agents and women’s inability to write and read) had negative association with years of schooling and the rest had negligible associations ranging from .01 to .09. The three selected independent variables (marital status: married, land ownership: other and barriers) significantly explained approximately 11% of the variance in educational needs of women farmers in the Northern Badia region of Jordan.

CONCLUSIONS AND IMPLICATIONS

From the analysis of the findings three major conclusions were drawn: 1) rural women’s highest educational needs are in livestock production, nutrition and resource management and marketing, 2) the perceived educational needs scores and the selected demographic characteristic of the rural women are independent of one another and 3) rural women’s outstanding barriers to Extension participation were 1) lack of information about Extension activities, 2) Extension agents do not often organize training programs for rural woman, 3) heavy loads of household task and time constraints, 4) permission by husband and 5) Extension training programs do not include woman's training needs.

Four items in the area of nutrition were among the top 12 ranked very high educational needs. Five are related to resource management and marketing of flocks

were the top 12 rank high educational needs. Educational courses should be planned that meet the identified needs of the rural women. Despite rural women’s valuable contribution in small ruminant production, they still have limited access to credit and land.

Rural women indicated a lack of knowledge in the area of milk processing techniques and controlling livestock diseases. From the above findings, Extension agents involved in planning programs must realize that rural women in Northern Badia of Jordan. District need education in the area of nutrition, resource management and marketing and livestock production. Extension program will be more effective as they focus on the educational needs of the rural women. One-quarter of respondents in this study had never attended school and the majority (49%) had only 1 to 8 years of schooling, indicating that rural women in Northern Badia of Jordan were a disadvantaged group of individuals, who have limited educational opportunities.

Women’s access to agricultural extension and their ability to comprehend and use technical information are lower when they lack education. More men than women are enrolled in training programs and gain more from developmental programs [14, 15]. Low investment in female education reduces productivity, efficiency and economic progress, inside and outside the household [16].

This research ranked educational needs for each item under the four-domain areas. This information can help Extension Departments related to the Ministry of Agriculture and the Badia Development Center in Jordan in placing their priorities on the items that were ranked high. Targeting planning will help meet the needs of rural women, attract a wider audience and lead to the success of Extension programs. Educational courses should be planned that meet the identified needs of the rural women, with emphasis given to those needs ranked highest.

ACKNOWLEDGEMENTS

The author would like to acknowledge the financial support and encouragement of Jordan Badia Research and Development Center, grant number 56/97.

REFERENCES

1. Sinn, R., J. Ketzis and T. Chen, 1999. The role of woman in the sheep and goat sector, *Small Ruminant Research*, 24: 259-269.

2. Valdivia, D., E. Dunn and J. Sherbourne, 1996. Gender, livestock and household peasant production: dairy and diversification in crop-livestock systems of an Andean Community. Small Ruminant. CRSP/IBTA Technical Report 33. University of Missouri, Colombia, USA.
3. Gidarakou, I., 1999. Young women's attitude towards agriculture and women's new roles in the Greek countryside: a first approach. *Journal of Rural Studies*, 15: 147-158.
4. Shortall, S., 2000. In and out of the milking parlour: a cross-national comparison of gender, the dairy industry and the state, *Women's Studies International Forum*, 23: 247-257.
5. Jointer, J., J. Sowe, E. Secka-Njie and L. Dempfle, 2001. Ownership pattern and management practices of small ruminants in the Gambia-implications for a breeding programme, *Small Ruminant Research*, 40: 101-108.
6. Lebbie, S.H.B., 2004. Goats under household conditions, *Small Ruminant Research*, 51: 131-136.
7. F.A.O., 1989. Guidelines on Communication for Rural Development. A Brief for Development Planners and Project Formulators. FAO, Rome.
8. M.O.A., 2004. Ministry of Agriculture. Report on Status of Women in Jordan. Amman, Jordan.
9. Salmen, L.F., 1999. The voice of the farmer in agricultural extension: a review of beneficiary assessments of agricultural extension and an inquiry into their potential as a management tool. AKIS Discussion Paper, World Bank, Washington.
10. Boldt, W.G., 1987. Targeting audiences and using creative media approaches. *Journal of Extension*, 25: 31-37.
11. Dutton, R.W., J.I. Clarke and A.M. Battikhi, 1998. *Arid Land Resources & their Management: Jordan's Desert Margin* (Eds.). Kegan Paul International Ltd., London and New York.
12. Borich, D.G., 1980. A needs assessment model for conducting follow-up studies. *Journal of Teacher Education*, 31: 39-41.
13. Travers, R.M.W., 1969. *An Introduction to Educational Research*. The MacMillan Company Collier-MacMillan Limited: London.
14. F.A.O., 1998. *The FAO Plan of Action for Women in Development. (1996-2001)*. Rome: FAO.
15. World Bank, 2000. *World Development Report 2000/2001*. Washington, D.C.: World Bank.
16. World Bank, 2000. *Engendering development: Through gender equality in rights, resources and voice*. Washington, D.C.: World Bank.

Compaction and Subsoiling Effects on Soil Properties, Plant Nutrient Concentration and Yield of Cotton (*Gossipium hirsutum* L.)

¹Waseem Raza, ²Sohail Yousaf, ³Younas Nadeem and ³Aslam John

¹College of Resource of Environmental Sciences, Nanjing Agriculture University, Nanjing, China

²Institutes of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

³Soil Chemistry Section, Ayub Agriculture Research Institute, Faisalabad, Pakistan

Abstract: Three hardpan levels; chisel broken hardpan, natural hardpan and artificial hardpan by compacting soil with 10 tone-loaded trolley, were developed to evaluate their effect on soil properties, nutrient uptake and yield of cotton, along with three levels of NPK fertilizers (half recommended, recommended and double recommended dose). The results revealed that natural hardpan and artificial hardpan caused yield reduction by 10 and 15% during the year 2004 and 9 and 14% during 2005, respectively. The maximum cotton yield during 2004 was obtained with two fold of recommended dose of NPK fertilizers that was not significant over yield with recommended dose of fertilizers. While during 2005, maximum cotton yield was obtained with recommended dose of fertilizers. Nutrient use efficiency, in case of recommended dose of NPK fertilizers was increased by 12 and 90% in the year 2004 and 23 and 94% in the year 2005 over half dose of recommended fertilizers and two fold of recommended dose of fertilizers, respectively. During 2004, hardpan broken with chiseling with double recommended dose of fertilizers gave maximum yield (3.28 t haG¹) which was non-significant with hardpan broken with chiseling with recommended dose of fertilizers and natural hardpan with recommended dose of fertilizers. During 2005, maximum yield of 2.9 t haG¹ was recorded with hardpan broken with chiseling with recommended dose of NPK fertilizers. The effect of hardpan and fertilizers was significant on plant leaf NPK concentration during both years except phosphorus concentration during 2005. Chisel broken hardpan with two fold of recommended fertilizers gave utmost plant leaf NPK concentration but it was non-significant with chisel broken hardpan with recommended dose of fertilizers.

Key words: Cotton % NPK Concentration % penetration resistance % subsoil compaction % yield

INTRODUCTION

Tillage refers to the different mechanical manipulations of the soil that are used to provide the necessary soil conditions favorable to the crop growth. A proper tillage can alleviate soil related constrains while improper tillage may lead to a range of degradative processes, e.g., deterioration in soil structure, accelerated erosion, depletion of soil organic matter and soil fertility and disruption in water cycles, organic carbon and plant nutrients [1]. Repeated use of tillage implements over the years created hardpan at about 15 cm depth. This hardpan influences bulk density, porosity and penetration resistance of soil which directly or indirectly affects the growth and yield of crops. Hardpan due to subsoil compaction of agricultural soils is a global concern due to adverse effects on crop yield and environment [2].

A number of studies have investigated the effects of root-restricting compacted soil layers on crop yield and the effects of subsoiling to shatter the compacted zones. Results are contradictory. The soils subsoiling resulted in an increase of cotton yields at two locations, did not affect yields at four locations and decreased yields at the remaining two locations [3]. The paraplowing effects soil physical properties for more than 2 yr but crop yield was not improved [4]. Fall chiseling with a paratill needed to be conducted annually to ensure minimizing the effects of soil compaction on crop growth [5]. In an experiment on barley (*Hordeum vulgare* L.), root length density in the upper 30 cm of soil and rooting depth decreased as the number of tractors passes increased from zero to six [6]. Bulk density and soil strength on traffic sides of a plant row can be much greater than those in the non-traffic side of the same row [7, 8]. Compaction can also result in low

water use efficiency [9] and less use of fertilizers [10]. In developing countries, tillage operations by farmers are generally performed with bullock and tractor to depth of 10-15 cm. Repeated use of tractor-driven cultivators creates a hardpan at about 15 cm depth which hinders the movement of water and air and inhibits growth of plant roots [11, 12]. So an experiment was planned to quantify the effects of tillage-induced hardpan on soil properties and crop growth.

MATERIALS AND METHODS

A two year study (2004-2005) was planned on research farms of Soil Chemistry Section, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan (31°26' North and 73°06' East). The soil at the experimental site is fine-loamy, mixed, *hyperthermic Typic haplargids*, covering 21% of canal irrigated area of the Punjab, Pakistan [13].

The soil had a natural hardpan (Bulk Density = 1.65 g cm³) at 15 cm depth was considered as control. To compare the effects of this hardpan with a soil having no hardpan, the natural hardpan was broken by chiseling (Bulk Density = 1.40 g cm³). An artificial hardpan of high bulk density (1.80 g cm³) was also created by removing the upper 15 cm soil and exposed soil surface was compacted with 10 tones load in a tractor driven trolley. The experiment was laid out in permanent plots following split plot design having three hardpan treatments in main plots with three replications and four fertilizer rates i.e. control (F0), half recommended doses (F1), recommended doses (F2) and two fold of recommended doses (F3) in the sub plots with three replications. Recommended fertilizer for cotton was 90-60-40 kg ha⁻¹, nitrogen (N), phosphorous (P) and potassium (K), respectively. Full dose of P, K and 1/2 N was applied at sowing time and remaining half N, 30 days after sowing. Area for main plot was 106m×105m and for sub plot was 26m×35m. Before sowing, seeds were acid delinted by Sulfuric acid @ 1L 10kgG¹ of seed. Sowing was done on 25th May with drill and flat sowing was converted into furrows before 1st irrigation. Thinning was completed before 1st irrigation within 20-25 days of planting and plant to plant distant, 6-9 inches and row-to-row distance, 30 inches was maintained. 1st irrigation was given after 30-40 days of planting. Subsequent irrigations were given 15-21 days intervals.

Before sowing composite soil samples were collected from 0-15 and 15-30 cm depth, air-dried, grounded and passed through 2 mm sieve. Soil samples were analyzed

Table 1: Physical and chemical characteristics of soil

Characteristics	Units	Depths	
		0-15 cm	15-30 cm
Physical			
Sand	g KgG ¹	405	406
Clay	g KgG ¹	282	285
Silt	g KgG ¹	312	304
Textural class	-	Sandy clay loam	Sandy clay loam
Bulk density	g cm ⁻³	1.41	1.68
Penetration resistance	Mpa	0.72	1.25
Chemical			
EC _e	dSmG ¹	0.82	0.72
pH _s	-	7.7	7.8
Organic matter	g KgG ¹	9.9	7.2
Kjeldhal-N	g KgG ¹	0.50	0.45
Olsen-P	mg KgG ¹	7.84	4.35
NH ₄ -OAc Ext. K	mg KgG ¹	176	128

for pH_s [14], electrical conductivity (EC_e) [15], organic matter [16], Olsen P [17], CH₃COONH₄ extractable K [18] and total N contents [19].

Physical properties of soil like textural class, bulk density (BD) and penetration resistance (PR) were also determined at the start of the study and after the harvest of each crop to see the changes brought about by different treatments. Soil bulk density and soil penetration resistance was measured by using the core method and cone penetrometer (30° cone tip angle, 9.2×10G³ m diameter), respectively [20].

At maturity, plant leaf samples were collected randomly from whole plant, oven dried at 70°C for 48 hours, grounded and digested in acid mixture (HNO₃ and HClO₄) and NPK concentrations were determined [19,21].

Cotton yield (seed + lint) was recorded and nutrient use efficiency (NUE) was calculated as below.

$$NUE = \frac{\text{yield with fertilizer (kg)} - \text{yield with out fertilizer (kg)}}{\text{Fertilizer nutrients applied (kg)}}$$

All the data were analyzed statistically for the analysis of variance technique [22]. The comparisons among the treatment means were made by Duncan's multiple range test [23].

RESULTS

Soil analysis: The results regarding physical properties of soil (Table 2) revealed that hardpan significantly affected the bulk density and penetration resistance. In

Table 2: Effect of hardpan on physical properties of soil

Treatments	Bulk density (mg/m ³)				Penetration Resistance (Mpa)			
	2004		2005		2004		2005	
	<i>at sowing</i>	<i>at harvesting</i>	<i>at sowing</i>	<i>at harvesting</i>	<i>at sowing</i>	<i>at harvesting</i>	<i>at sowing</i>	<i>at harvesting</i>
HP0	1.42 c	1.61 b	1.63 b	1.66 b	0.67 c	0.73 b	0.75 c	0.78 b
HP1	1.65 b	1.63 b	1.64 b	1.65 b	1.11 b	1.09 a	1.06 b	1.09 a
HP2	1.84 a	1.79 a	1.78 a	1.76 a	1.28 a	1.25 a	1.26 a	1.19 a
LSD	0.0717				0.2028			

Means sharing same letter don't differ significantly (P=0.05)

HP0= Natural hardpan broken with chiseling, HP1= Natural hardpan, HP2 = Artificial hardpan

Table 3: Hardpan effects on Cotton yield

Tr. no.	Treatments	Cotton yield (t haG ¹) (seed + lint)	
		2004	2005
1	Natural hardpan broken by chiseling (HP0)	2.83 a	2.55 a
2	Natural hardpan (HP1)	2.56 b	2.33 b
3	Artificial hardpan (HP2)	2.40 c	2.21 c
LSD	0.09	0.07	

Table 4: Fertilizer effects on Cotton yield and nutrient use efficiency

Tr. no.	Treatments	Cotton yield (t haG ¹) (seed + lint)		Nutrient use (kg Cotton yield efficiency /kg nutrient)	
		2004	2005	2004	2005
1	Control (F0)	2.04 c	1.93 d	-	-
2	½ recommended dose of NPK (F1)	2.44 b	2.52 b	2.42	1.58
3	Recommended dose of NPK (F2)	2.93 a	2.64 a	2.70	1.94
4	2 × recommended dose of NPK (F3)	2.98 a	2.33 c	1.41	1.00
LSD	0.15	0.08			

Table 5: Hardpan and Fertilizer effect on yield of Cotton (t haG¹)

Treatments	2004				2005			
	F0	F1	F2	F3	F0	F1	F2	F3
HP0	2.22 f	2.62 de	3.21 a	3.28 a	2.00 f	2.67 b	2.90 a	2.53 cd
HP1	1.88 I	2.38 efg	3.04 ab	2.92 bc	1.98 f	2.45 d	2.61 bc	2.31 e
HP2	2.03 hi	2.31 fg	2.52 def	2.71 cd	1.80 g	2.53 cd	2.43 d	2.22 e
LSD	0.26				0.10			

Means sharing same letter don't differ significantly (P=0.05)

comparison with natural hardpan, breaking hardpan with chisel plough reduced the bulk density and penetration resistance by 11 and 42% while artificial hardpan increased these by 10 and 10.4%, respectively. During two years of study (2004-2005) bulk density and penetration resistance of same treatment remained the same.

Yield and nutrient use efficiency: The data (Table 3, 4 and 5) indicated that natural hardpan and artificial

hardpan caused yield (seed + lint) reduction by 10 and 15% during the year 2004 and by 9 and 14% during 2005, respectively. All the fertilizer rates produced statistically higher yield than that of control treatment. The maximum cotton yield of 2.98 t haG¹ during 2004 was obtained with two fold of recommended dose of NPK fertilizers (F3) that was not significant over yield with recommended dose of fertilizers (F2). While during the year 2005, maximum cotton yield of 2.64 t haG¹ was obtained with

Table 6: Effect of hardpan on nutrient concentration in Cotton leaf

		2004				2005			
Treatments		F0	F1	F2	F3	F0	F1	F2	F3
N g/kg	HP0	40.2 ef	43.7 ab	43.3 b	44.0 ab	40.5 e	45.6 bc	46.2 ab	46.8 a
	HP1	40.1 ef	41.9 cd	43.9 ab	44.4 a	41.0 e	45.0 c	45.1 c	45.8 abc
	HP2	39.6 f	40.3 ef	41.0 de	42.1 c	38.7 f	43.9 d	44.9 c	45.4 bc
LSD	0.10					0.098			
P g/kg	HP0	4.1 cde	4.9 ab	5.1 a	4.9 ab	3.9	4.1	4.5	4.6
	HP1	4.0 de	4.8 abc	4.9 ab	4.6 abc	3.8	3.9	4.2	4.5
	HP2	3.8 e	4.5 bcd	4.7 bcd	4.8 abc	4.0	3.9	4.1	4.3
LSD	0.07					NS			
K g/kg	HP0	30.2 e	30.4 de	31.0 cd	33.1 a	24.7 ab	28.5 ab	29.6 a	29.9 a
	HP1	28.9 f	30.1 e	30.2 e	32.5 a	24.9 ab	29.6 a	27.6 ab	28.4 ab
	HP2	26.5 g	31.8 b	31.5 bc	31.6 bc	23.4 b	25.2 ab	26.1 ab	26.4 ab
LSD	0.07					0.06			

Means sharing same letter don't differ significantly (P=0.05)

recommended dose of fertilizers (F3). Nutrient use efficiency (NUE), at the recommended dose of NPK fertilizers (F2) was increased by 12 and 90% in the year 2004 and 23 and 94% in the year 2005 over half dose of recommended fertilizers (F1) and two fold of recommended dose of fertilizers (F3), respectively. After control treatment, minimum cotton yield (2.44 t haG¹) during 2004 was recorded with half recommended dose of NPK fertilizers (F1) and during the year 2005, minimum cotton yield (2.33 t haG¹) was recorded with two fold of recommended dose NPK fertilizers (F3). Minimum nutrient use efficiency of 1.41 kg cotton yield per kg nutrient during 2004 and 1 kg cotton yield per kg nutrient during 2005 was obtained with two fold of recommended dose of NPK fertilizers. Hardpan and fertilizer interaction was found significant in both years. During the year 2004, hardpan broken with chiseling with double recommended dose of fertilizers (F3) gave maximum yield (3.28 t haG¹) which was non-significant with chisel broken hardpan with recommended dose of fertilizers and natural hardpan with recommended dose of fertilizers. During 2005, maximum yield of 2.9 t haG¹ was recorded with chisel broken hardpan with recommended dose of NPK fertilizers. The lowest yield of 1.9 t haG¹ during 2004 and 1.8 t haG¹ during 2005 was produced under natural and artificial hardpan where no fertilizer was applied, respectively.

Chemical composition of cotton leaf: Results presented in Table 6 regarding NPK concentration in cotton leaf at flowering stage revealed that during the year 2004, maximum nitrogen concentration (4.4%) was recorded from natural hardpan where double recommended dose of

fertilizer was applied which was non-significant with natural hardpan where recommended dose of fertilizers was applied and with hardpan broken by chiseling where two fold of recommended and half of recommended dose of fertilizer was used, respectively. Maximum phosphorus concentration (0.51%) was recorded from hardpan broken by chiseling with recommended dose of fertilizer while it was non-significant with all other treatments except treatments where no fertilizer was used. Hardpan broken by chiseling with two fold of recommended fertilizer gave utmost potassium concentration (3.25%) that was non-significant with natural hardpan where two fold of recommended dose of fertilizer was applied. During the year 2005, maximum nitrogen concentration (4.68%) was obtained from hardpan broken by chiseling with two fold of recommended dose of fertilizer which was non-significant with hardpan broken by chiseling where recommended dose of fertilizer was practiced and natural hardpan where two fold of recommended dose of fertilizer was used. There was non-significant effect of hardpan and fertilizer levels on phosphorous concentration in cotton leaf during 2005. Maximum potassium concentration was obtained from hardpan broken by chiseling where two fold of recommended dose of fertilizer was used but it was non-significant with all treatments except, artificial hardpan where no fertilizer was practiced.

DISCUSSION

Conventional cotton production practices in developing countries involve several shallow tillage operations that lead to hardpan formation in subsoil

region. Site used for this experiment also has hardpan at 15 cm depth. We created an artificial hardpan and broke natural hardpan by chiseling to compare its effect on cotton.

Data in Tables 3, 4 and 5 indicated that natural hardpan and artificial hardpan caused the yield reduction by 10 and 15% during the year 2004 and 9 and 14% during 2005, respectively and higher rates of fertilizer were not able to overcome hardpan constrains. This decrease in crop yield due to subsoil compaction may be partially a result of low nutrient and water uptake and availability under compacted soil conditions [9]. Physical conditions detrimental to root proliferation in subsoil are frequently related to hardpans that develop below plough layer and higher levels of fertilizers cannot overcome hardpan constrains [24, 25]. This hardpan has high bulk density, high penetration resistance, reduced soil aeration, few macropores for roots to grow through and mechanical impedance great enough to markedly reduce root growth rates [11, 12]. Our results are also supported by previous studies [26, 27].

Data regarding the effect of hardpan and fertilizers on NPK concentration in cotton leaf (Table 6) indicated that double recommended dose of fertilizer increased uptake but it was nonsignificant with recommended dose of fertilizer while half dose of fertilizers decreased cotton leaf NPK contents. Similar results are reported by some scientists [9, 28]. Reduction in soil water availability due to decrease water infiltration, less volume of soil explored by the roots and anatomical and morphological changes in the root system and a small portion of macro pores in compacted soil may account for lower NPK concentration in plant leaf. Several studies have documented increased rates of denitrification or N₂O production in compacted soils but other losses may also occur through increased surface runoff in compacted soils due to lower water infiltration [29, 30]. According to literature, deep tillage (subsoiling) of certain clayey soils in the fall when the soil profile is dry significantly increases yields and net returns from this production system [31]. For optimum yields, many soils require deep tillage as a method of alleviating soil compaction after every three years. However, this tillage event can be costly. An experiment can be conducted to determine if mapping the layer of soil compaction and then delivering tillage to the exact depth of soil compaction may reduce tillage power requirements while maintaining cotton yields. Average cotton yields over this three-year period showed that site-specific tillage produced yields equivalent to those produced by the uniform deep tillage treatment while requiring 27% less tillage power [32]. Continued development of technology

and equipment necessary for site-specific tillage could contribute to a more energy efficient food production system.

CONCLUSIONS

Subsoil compaction, whether natural or induced by vehicle traffic, reduces crop yield and quality because of several factors. The severity of subsoil compaction artificially created in this experiment may not occur in traditional small-scale farming practices, the potential of severe subsoil compaction in alluvial soils exists with progressive increase in mechanization of farm operations in Punjab and elsewhere in world. Therefore, appropriate measures such as periodic chiselling; controlled traffic, conservation and site specific tillage and incorporation of crops with deep tap root systemic rotation cycle are necessary to minimize the risks of subsoil compaction.

ACKNOWLEDGEMENTS

The authors want to thank Aslam John (ARO, Ayub Agriculture Research Institute, Faisalabad, Pakistan) for his cooperation and technical help regarding experiment. Further, authors owe thanks to Abid Niaz, Khalid Rashid (AARI, Faisalabad, Pakistan) and Sohail Yousaf (UAF, Faisalabad, Pakistan) for their guidance.

REFERENCES

1. Lal, R., 1996. Axle load and tillage effects on crop yields on a Mollic Ochraqualf in northwest Ohio. *Soil Tillage and Research*, 37: 143-160.
2. Hakansson, I. and R.C. Reeder, 1994. Subsoil compaction by vehicles with high axle load-extent persistence and crop response. *Soil Tillage and Research*, 29: 277-304.
3. Taylor, H.M. and R.R. Bruce, 1968. Effects of soil strength on root growth and crop yield in the southern United States. In: *Trans. 9th International Congress of Soil Science, Adelaide, Australia. International Society of Soil Science and Angus and Robertson LTD, Sydney, NSW, Australia*, pp: 803-811.
4. Tollner, W.E., W.L. Hargrove and R.L. Clark, 1987. Paraplowing a piedmont soil for improving crop performance. p. 327-331. In: *Optimum erosion control at least cost. Proc. Natl. Symp. on Conserv. Syst., Chicago, IL. 14-15 Dec. 1987. ASAE, St. Joseph, MI.*

5. Clark, R.L., D.E. Radcliffe, G.W. Langdale and R.R. Bruce, 1993. Soil strength and water infiltration as affected by paratillage frequency. *Transaction of the ASAE*, 36: 1301-1306.
6. Willatt, S.T., 1986. Root growth of winter barley in a soil compacted by the passage of tractors. *Soil Tillage and Research*, 7: 41-50.
7. Kaspar, T.C., H.J. Brown and E.M. Kassmeyer, 1991. Corn root distribution as affected by tillage, wheel traffic and fertilizer placement. *Soil Science Society of America Journal*, 55: 1390-1394.
8. Voorhees, W.B., 1992. Wheel-induced soil physical limitations to root growth. In: *Limitations to Plant Root Growth*, Eds. Hatfield A.L. and B.A. Stewart. *Advances in Soil Science*, 19: 73-95.
9. Ishaq, M., M. Ibrahim, A.Hassan, M. Saeed and R. Lal, 2001. Subsoil compaction effects on crops in Punjab, Pakistan. II. Root growth and nutrient uptake of wheat and sorghum. *Soil Tillage and Research*, 60: 153-161.
10. Stepniewski, W. and G. Przywara, 1992. The influence of soil oxygen availability on yield and nutrient uptake by winter rye (*Secale cereale*). *Plant Soil*, 143: 267-274.
11. Hassan, M.M. and P.J. Gregory, 1999. Water transmission properties as affected by cropping and tillage systems. *Pakistan Journal of Soil Science*, 16: 29-38.
12. Rafiq, M., 1990. Soil variability in agronomic research. *Pakistan Journal of Soil Science*, 5: 9-14.
13. Anonymous, 1980-2000. Reconnaissance Soil Survey Report of Punjab Areas. *Soil Survey of Pakistan*, Lahore.
14. McLean, E.O., 1982. Soil pH and lime requirement. In: *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. Agronomy Monograph No. 9, 2nd Edition, Eds., Page, A.L., R.H. Miller and D.R. Keeney. Soil Science Society of America (SSSA) Madison, WI, pp: 199-224.
15. Rhoades, J.D., 1982a. Soluble salts. In: *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. Agronomy Monograph no. 9, 2nd Edition, Eds., Page, A.L., R.H. Miller and D.R. Keeney. Soil Science Society of America (SSSA) Madison, WI, pp: 167-179.
16. Nelson, D.W. and L.E. Sommers, 1982. Total carbon, organic carbon and organic matter. In: *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. Agronomy Monograph no. 9, 2nd Edition, Eds., Page, A.L., R.H. Miller and D.R. Keeney. Soil Science Society of America (SSSA) Madison, WI, pp: 539-579.
17. Olsen, S.R. and L.E. Sommers, 1982. Phosphorus. In: *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. Agronomy Monograph no. 9, 2nd Edition, Eds., Page, A.L., R.H. Miller and D.R. Keeney. Soil Science Society of America (SSSA) Madison, WI, pp: 403-430.
18. Knudsen, D., G.A. Peterson and P.F. Pratt, 1982. Lithium, sodium and potassium. In: *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. Agronomy Monograph no. 9, 2nd Edition, Eds., Page A.L., R.H. Miller and D.R. Keeney. Soil Science Society of America (SSSA) Madison, WI, pp: 115-246.
19. Tecator, A.B., 1991. Determination of Kjeldhal nitrogen content with Kjeltec autosystems I, II, III and IV. Tector application note AN 30/81, Hoganas, Sweden.
20. Blake, G.R. and K.H. Harthe, 1986. Bulk density. In: *Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods*, Agronomy Monograph No. 9, 2nd Edition, Ed., A. Klute. Soil Science society of America (SSSA) Madison, WI, pp: 363-375.
21. Yoshida, S., D.A. Forno, J.H. Cock and K.A. Gomez, 1976. *Laboratory manual of physiological studies of Rice*. IRRI, Los Banos, Philippines.
22. Gomez, K.A. and A.A. Gomez, 1984. *Statistical procedures for Agricultural Research*, 2nd Edition. Wiley, New York. pp: 680.
23. Duncan, B.D., 1955. Multiple range and multiple *F*-test. *Biometrics*, 11: 1-42.
24. Gill, K.S. and B.S. Aulakh, 1990. Wheat yield and bulk density response to some tillage systems on an Oxisol. *Soil Tillage and Research*, 18: 37-45.
25. Maurya, P.R., 1988. Performance of zero tillage in wheat and maize production under different soil and climate conditions in Nigeria. In: *Proc. 11th Conf. Int' I Soil Tillage Research Organization*, Vol. 2. ISTRO, Edinburgh, UK, pp: 769-774.
26. Al-Adawi, S.S. and R.C. Reeder, 1996. Compaction and subsoiling effect on corn and soybean yields and soil physical properties. *Transactions of the ASAE*, 39: 1641-1649.
27. Alakukku, L. and P. Elonen, 1994. Finish experiment on subsoil compaction by vehicles with high axle load. *Soil Tillage Research*, 29: 151-155.
28. Ishaq, M., M. Ibrahim and R. Lal, 2003. Persistence of subsoil compaction effects on soil properties and growth of wheat and cotton in Pakistan. *Experimental Agriculture*, 39: 341-348.

29. Sitaula, B.K., S. Hansen, J.I.B. Sitaula and L.R. Bakken, 2000. Effects of soil compaction on N₂O emission in agricultural soil. *Chemosphere – Global Change*, 2: 367-371.
30. Torbert, H.A., K.N. Potter and J.E. Morrison jr, 2001. Tillage system fertilizer nitrogen rate and timing effect on corn yields in the Texas Black land Prairie. *Agronomy journal*, 93: 1119-1124.
31. Richard, A., Wesley, A. Lowrey, Smith, R. Stan and Spurlock, 2000. Residual effects of fall deep tillage on soybean yields and net returns on tunica clay soil. *Agronomy Journal*, 92: 941-947.
32. Raper, R.L., D.W. Reeves, J. Shaw, E. Van Santen, P. Mask and T.E. Grift, 2003. Effect of site-specific tillage on draft requirements and cotton yield. *Proceedings of the 2003 Beltwide Cotton Conference*. Nashville, TN., pp: 7-11.5.

Olea europaea L. A Botanical Contribution to Culture

Sophia Rhizopoulou

National and Kapodistrian University of Athens, Department of Biology,
Section of Botany, Panepistimioupoli, Athens 157 84, Greece

Abstract: One of the oldest known cultivated plant species is *Olea europaea* L., the olive tree. The wild olive tree is an evergreen, long-lived species, wide-spread as a native plant in the Mediterranean province. This sacred tree of the goddess Athena is intimately linked with the civilizations which developed around the shores of the Mediterranean and makes a starting point for mythological and symbolic forms, as well as for tradition, cultivation, diet, health and culture. In modern times, the olive has spread widely over the world.

Key words: *Olea* % etymology % origin % cultivation % culture

INTRODUCTION

Olea europaea L. (Fig. 1 & Table 1) belongs to a genus of about 20-25 species in the family Oleaceae [1-3] and it is one of the earliest cultivated plants. The olive tree is an evergreen, slow-growing species, tolerant to drought stress and extremely long-lived, with a life expectancy of about 500 years. It is indicative that Theophrastus, 24 centuries ago, wrote: 'Perhaps we may say that the longest-lived tree is that which in all ways, is able to persist, as does the olive by its trunk, by its power of developing sidegrowth and by the fact its roots are so hard to destroy' [4, book IV.13.5]. The most ancient traces of *Olea* are fossilised leaves, found on the island of Santorini in the Aegean Archipelago, dating back 50,000-60,000 years [5, 6]. Olive cultivation originated in a valley of the river Jordan in the Eastern Mediterranean area [7] and has a history as long as that of western civilization [8, 9].

Sophocles (5th century BC) wrote a hymn to the olive tree, for his last play Oedipus at Colonos (401 BC):

- ⊘ There is a plant unheard of in the fabulous land of Asia,
- ⊘ unknown to Doric earth - a thing immortal;
- ⊘ gift of a goddess, beyond the control of hands,
- ⊘ tough, self-renewing, an enduring wealth,
- ⊘ passing through generations
- ⊘ the invincible grey-leafed olive.
- ⊘ Aged survivor of all vicissitudes,
- ⊘ it knows protection of the all-seeing eye of Zeus,

Table 1: Classification of *Olea europaea*

Superdivision	Spermatophyta-seed plants
Division	Magnoliophyta-flowering plants
Class	Magnoliopsida-dicotyledons
Subclass	Asteridae-
Order	Scrophulariales-
Family	Oleaceae-olive family
Genus	<i>Olea</i> -olive
Species	<i>Olea europaea</i> L. -olive

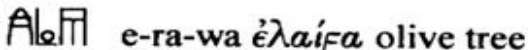
 *e-ra-wa ἐλαίφα olive tree*

Fig. 1: *Olea europaea* L., of the Linnean herbarium (microfiche No: IDC 4.3, Department of Phanerogamic Botany Swedish Museum of Natural History)

- ⊘ whose sunlight always regards it,
- ⊘ and of grey-eyed Athena.

The purpose of this study is to foster greater understanding of the botanical, historical and philological evidence for the origin and the distribution of the olive tree, 'the queen of all trees' according to Columella (Libri De Re Rustica, 42 AD).

Botanical ancestor of *Olea*: The botanical ancestor of the cultivated *Olea europaea* L. is believed to be a group of wild olives traditionally called *oleaster* olives. Over large areas in the Mediterranean province, *oleasters* thrive as a constituent of *maquis* formations, within a

climatic region that has been defined by Köppen [10], as the *olive climate* [11-13]. Wild olive trees have been treated by some botanists as an independent species, i.e. *Olea oleaster* Hoffm. & Link., but because of their close morphological and genetic affinities to the cultivated tree, most researchers dealing with Mediterranean plants today regard *oleaster* as the wild race of the cultivated species and place it within *Olea europaea* L., either as a subspecies [subsp. *oleaster* (Hoffm. & Link) Hegi], or as a variety [var. *sylvestris* (Mill.) Lehr. = var. *oleaster* (Hoffm. & Link.) DC.] [2]. Theophrastus (371-286 BC) stated that the wild olive tree differs from the cultivated olive in having spiny lower branches [14, book V.12.8] and small leaves and drupes [4, book I.14.2 & book II.2.12]. Cultivated and wild olive trees possess similar characteristics and it is difficult to distinguish stands of wild olive trees [15-17]. According to archaeological evidence, *Olea* was cultivated in Crete and Syria, as long ago as 2,500 BC [18, 19]. Genetic studies support the hypothesis that olive domestication occurred in many locations in the Mediterranean basin [20]. More recent work indicates that there must have been a period of 'pre-domestication cultivation' in which the wild ancestors of domestic species were intentionally cultivated [21, 22]. The dimensions of carbonised stones of *Olea europaea* have proved to be an invaluable parameter that can be used to distinguish between wild and cultivated varieties [23-25].

Olea europaea is a hermaphrodite tree that blossoms in spring; two kinds of small, fragrant, cream-coloured flowers produce pollen, the species being largely wind pollinated, though, most olive varieties are self-fertile. The fruit of the olive tree is a drupe that usually changes colour from green to purple or nearly black, when fully ripe in late autumn and the oil is expressed by the fleshy pericarp. Pliny (23-76 AD) refers to *Historia Naturalis* (XII.14, XII.60, XV.1-7, XXIII.34-60) fifteen varieties of olives and the usefulness of olive oil. The varieties known to the modern farmer are numerous, because olive trees have been exclusively cross-pollinated. The cultivars vary considerably in size, shape, oil content and flavour and molecular markers have been used to classify them [26, 27].

The cultivation of olive trees has been expanded to Egypt, France Iberia, Israel, Italy, Lebanon, Morocco and Tunis. Olive trees have been introduced to Chile, the Caribbean, Peru, Argentina, Brazil, Mexico and finally, in the 17th century, to California. The olive tree has also been introduced into Chinese agriculture and it grows vigorously in South Australia and some parts of



Fig. 2: *Olea*, in Linear B (i.e. the first Greek writing system) used between 14th and 12th century BC.

South Africa. *Olea europaea* is now considered as an edible, medicinal and useful plant for a healthier world. It is noteworthy that olive oil contains 14.8% saturated fat and 85.2% unsaturated fat [28] and it is valued as an important item of diet [29]. Virgin olive oil, identified by its delicate and unique aroma [30], is highly appreciated by consumers, because it is consumed in its crude form without any refining process.

Etymology of *Olea*: The first word for *Olea* appeared in Linear B (Fig. 2), on clay tablets found in Greece dated to the 13th century BC [31, 32]. The word *olive* and all the surviving forms are derived from the Greek word *elaa* (g8V"), according to Theophrastus) and *ela*• (g8""") [33]. Thus, we have *alev* in Gothic, *olia* in old Scandinavian, *al* (oil) in Anglo-Saxon, *olei* (öl) in old High German and *olea*, *oliva*, *oleum*, *olivum* in Latin [34]. The Semitic word *zeit* for *Olea* is encountered in the Arabic *zenboudje* (wild olive) and *zitoun* (cultivated olive), in the Andalusian *azenbucha*, in the Portuguese *zambugeiro*, in the word *zayit* in Israel and *zuttin* in Morocco; it is interesting to note that among the Tuaregs the wild olive is called *aleo* [32].

It would seem that a relationship in definition between the word for oil (*éleon*) and the word for mercy (*éleos*) in Greek, might be due to a false etymology [35]. However, in dramatic masterpiece-texts such as *Oedipus Rex* of Sophocles (434 BC) a person who came in supplication, seeking mercy and understanding from his follow-men, when he had committed a grave offence, holds olive branches in his hands. In *Eumenides* of Aeschylus (5th century BC), the soothsayer Pythia of the Delphi oracle announces 'I see a man with bloody hands seated at the Navel, postured in the suppliant's seat, holding a fresh stem of olive'. Olive trees were closely planted in Delphi valley from very ancient times.

In the Shakespeare's Twelfth Night of (I.5.204) 'I bring no overture of war, no taxation of homage, I hold the olive in my hand: my words are as full of peace as matter'. Therefore, it is likely that olive branch has been a symbol of peace and of the reconciliation of man with man. In this spirit, olive branches appear in the flag of the United Nations Organization and have become a symbol of longevity, purification, strength, prosperity, wisdom, victory and peace. It is well known that in the Olympic games, the winners were crowned with wreaths made of olive branches [36].

***Olea* in the (early) civilized mediterranean province:**

Olea europaea appears in the Bible and in the Qur'ân, as the most sacred, most revered and most adored tree, playing an important role in civilization, religion, diet and art. *Olea* was cultivated during the early Biblical period for the fruits from which precious oil was extracted, while olives were treated with pickling and salting techniques for domestic and export purposes [37]. Olive trees have been cultivated along and above the 15° isotherm providing a useful substitute for the butter and animal fats consumed by the races of the north. Hence, the olive became an emblem of national wealth and domestic plenty [19]. 'The whole Mediterranean seems to emerge from the pungent taste of black olives, a taste older than that of meat and wine; a primeval taste, like the taste of water' [38].

The species was known in ancient Egypt, as is shown by papyri (1,550 BC), mummies crowned with olive wreaths and a hymn of Ramesses III (1198-1176 BC) to the god Ra (the sun): 'I have planted many olive trees in gardens, in the city of Heliopolis; from these plants come a very pure oil to keep alight the lamps of your altar' [39]. In the *Iliad* and *Odyssey* of Homer (written before 700 BC), olive oil provides extensive power when used in ritual anointing. The species was, also, known in Armenia [1]. Roman people employed it largely in food and cookery; in the luxurious days of the later empire it became a favourite axiom that long and pleasant life depends on two fluids 'wine within and oil without' [40, book XXIV.150]. Strabo the geographer (63 BC-23 AD) and Columella in his *Libri De Re Rustica* (42 AD) mentioned the quality of Spanish oil.

***Olea* from mythology to the early history of plants:** The city of Athens was named after the goddess Athena, who brought the olive tree to the city. When Athena won the contest against Poseidon for the patronage of the city, an olive sprang from the barren rock of Acropolis at the

bidding of the goddess. That this myth has some relation to the first planting of the olive tree in Greece seems certain according to Herodotus (485-425 BC, *Epidaurians*). In fact, Theophrastus writes that olive-wood is more apt than other woods to produce shoots even when lying idle or made into manufactured articles; this it often does, if it obtains moisture and lies in a damp place [4, book V.9.8]. The olive tree long stood on the Acropolis and, though destroyed in the Persian invasion (480 BC), sprouted again from its root [41]. To the long-lived character of *olea*, both cultivated and wild witness is born also by the tales handed down in mythology, as the olive at Athens [4, book IV.13.2]. Aristotle (384-322 BC) tells us that even the death penalty could be imposed on a person who uprooted or destroyed a sacred olive tree. Those trees, later totally twisted (hunchbacked), being extremely old, were growing in the Academy, still in existence at the time of Pausanias (2nd century AD). It appears that the life of the individual olive (in regard to which one should make the trunk the essential part and standard in estimating the time) lasts for about two hundred years [4, book IV.13.5].

The town of Athens was surrounded by extensive olive groves down to the times of Ottoman rule, as was witnessed by travellers [42-44]; among them John Sibthorp (Professor of Botany in Oxford, 1784-1796), Sir J. E. Smith (the first president of the Linnean Society) and J. Lindley (a great East Anglian botanist) passed through a venerable forest of olives during a trip to Athens, in 1787 [45, 46]. Excavation of the Athenian Agora (1931-1970) has uncovered evidence for abundant olive plants in antiquity [47].

Material evidence of the extent of olive oil trade is plentiful. Thousands of small oil lamps have been found in Greece, during the Bronze Age (2800-1100 BC). In the first stepped Pyramid (known as the Mastaba of Sakkara), a representation of one of the earliest known oil presses exists. In the palace of Minos in Crete an olive press was in operation (2000-1000 BC). The Athenian pottery industry was stimulated largely by the demand for containers in which olive oil was exported. Oil of the sacred trees was put into black-figure amphorae, decorated with olive-harvest motives. Perfumers put their odours in oil [14, book VI.19.3] and small vessels with scented olive oil were one of the most favourite love gifts [5]. Oil in phials was used as a cleanser for the body, just like soap nowadays [18]. Samples of oil vessel (lekythos) with a depiction of a siren in front of an olive branch are exhibited in Museums and Art Galleries.

***Olea europaea* as an inspiration in art and research:**

From myth into history and from there into art and research, *Olea europaea* has come to occupy a dominant place in our lives peacefully. Olive trees, features of the Mediterranean landscape, have inspired artists, who tried to capture the emerald and silver hues of the leaves shimmering against an azure Mediterranean sky or the gnarled and twisted branches that withstand the ages. In Italy, Poliphilio in *Hypnerotomachia* [48], during a fantastic journey of love through gardens, found himself in a dream 'I was encircled by pleasant hills of no great height with wild olives disposed according to the aspect of forested slopes'. In Iran, nearly four centuries later (1994), an olive grove is magnified as a place of desire and unsatisfied meeting, in the multi-awarded film of Abbas Kiarostami entitled 'Through the olive tree'. Impressionists were especially enamoured of the beauty of olive trees, which were vigorously painted by Vincent van Gogh (1853-1890). Writing to his brother Theodore (letter 587, April 1889), van Gogh stated 'I am struggling to capture the light of the olives. It is silver, sometimes bluish, sometimes greenish, off-white, on a ground of yellow, pink, violet, or orange to red ochre. It is very difficult' [49]. A couple of months later (letter 595, June 1889), Vincent declared: 'At last, I have a landscape with olive trees'.

Olea europaea has evolved a number of adaptive mechanisms to survive the prolonged summer-drought conditions in the Mediterranean environment, which affect water status and CO₂ assimilation [50, 51]. Its leaves expand within three months, during spring and are replaced after a two-year life period [52, 53]; a second growth flush occurs in autumn [54]. Their capacity to undergo dehydration is limited by a high internal diffusive resistance, which is due to the dense packing of mesophyll cells [55, 56]. A layer of peltate scales on the abaxial leaf surface may intercept incoming irradiation and impede the diffusion of CO₂ into the leaf. These scales are likely to function by trapping warm moist air below the stomatal aperture and consequently reducing water loss from the plant [57-60]. The species has been studied as a predictor of climate change [61]. Its stomatal density, investigated in leaf-samples originating from Tutankhamun's tomb (1327 BC) and from material dating to 332 BC, 1818 and 1978 AD, was used as an indicator of the effect of rising, atmospheric CO₂ levels in leaf structure and function [62, 63]. Recently, airborne pollen concentration, reflecting the flower phenology of olive populations within a radius of 50 km, has been considered as a sensitive indicator of climatic warming [64-66]. Yet

from the era of Greek and Egyptian civilizations, in which the olive tree was a divine gift for the mortals [67], to the century of globalisation, *Olea europaea* remains a symbolic element of plenty, peace and serenity [37].

ACKNOWLEDGEMENTS

I wish to thank Prof. P. Valavanis for comments on literature and Danae Koukos for comments on an earlier draft of the manuscript.

REFERENCES

1. Moazzo, G.P., 1994. Les plantes d' Homère et de quelques autres poètes de l' antiquité: V. L' Olivier (Elaie). Ann. Musei Goulandris, 9: 185-233.
2. Zohary, D. and M. Hopf, 2000. Domestication of plants in the Old world. Clarendon Press.
3. Riley, F.R., 2002. Olive oil production on Bronze Age Crete: nutritional properties, processing methods and storage life of Minoan olive oil. Oxford J. Archaeol., 21: 63-75.
4. Theophrastus, 1926. Fourth century B.C. Enquiry into plants (vol. I, II) in ancient Greek. Hort AF (translator) 1926. Loeb classical library. Cambridge: Heinemann London and Harvard University Press.
5. Valavanis, P., 2004. Olive oil and the ancient Greeks. In Ode to the olive tree. Academy of Athens, pp: 62-73.
6. Velitzelos, E. and D. Velitzelos, 2005. Geohistorical evidence on the evolution of plants in the Aegean Sea. In Biodiversity and natural heritage in the Aegean, Eds., Karamanos, A.J. and C.A. Thanos. The Agricultural University of Athens, pp: 133-148.
7. Zohary, D. and P. Spiegel-Roy, 1975. Beginnings of fruit growing in the old world. Science, 187: 319-327.
8. Forbes, H. and L. Foxhall, 1978. The queen of all trees. Preliminary notes on the archaeology of the olive. Expedition, 21: 37-47.
9. Oliveira, J., R.M. Tavares and H. Gerós, 2002. Utilization and transport of glucose in *Olea europaea* cell suspensions. Plant Cell Physiol., 43: 1510-1517.
10. Köppen, W., 1923. Die Klimate der Erde. De Gruyter.
11. Specht, L. and J.E. Moll, 1983. Mediterranean-type heathlands and sclerophyllous shrublands of the world: an overview. In Mediterranean-type ecosystems, Eds., Kruger F.J., D.T. Mitchell and J.U.M. Jarvis, Srpinger-Verlag, pp: 41-65.

12. Tutin, T.G., V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters and D.A. Webb, 1992. *Flora Europaea*. Cambridge University Press, 3: 55.
13. Naveh, Z. and A. Lieberman, 1993. The evolution of the Mediterranean landscapes. In *Landscape Ecology*, Eds., Naveh, Z. and A. Lieberman, Springer-Verlag, pp: 256-265.
14. Theophrastus, 1976. Fourth century B.C. *De Causis Plantarum* (vol. I-VI) in ancient Greek, Einarson B and Link GKK (translators), 1976. Loeb classical library. Heinemann London and Harvard University Press.
15. Greuter, W., 1972. The relict element of the flora of Crete and its evolutionary significance. In *Taxonomy, Phytogeography and Evolution*, Ed., Valentine, D.H., Academic Press, pp: 161-167.
16. Liphshitz, N., R. Gophna, M. Hartman and B. Gideon, 1991. Beginning of olive (*Olea europaea*) cultivation in the Old World: a reassessment. *J. Archaeological Sci.*, 18: 441-453.
17. Terral, J.F., 1996. Wild and cultivated olive (*Olea europaea* L.): A new approach to an old problem using inorganic analyses of modern wood and archaeological charcoal. *Rev. Palaeobot. and Palynol.*, 1: 383-307.
18. Boardman, J., 1976. The olive in the Mediterranean: its culture and use. *Philosophical Transactions of the Royal Society London B*, 275: 187-196.
19. Terral, J.F., N. Alonso, R.B. Capdevila, N. Chatti, L. Fabre, G. Fiorentino, P. Marinval, G.P. Jord•, B. Pradat, N. Rovira and P. Alibert, 2004. Historical biogeography of olive domestication (*Olea europaea* L.) as revealed by geometrical morphometry applied to biological and archaeological material. *J. Biogeogr.*, 31: 63-77.
20. Besnard, G., B. Khadari, P. Baradat and A. Bervillé, 2002. Combination of chloroplast and mitochondrial DNA polymorphisms to study cytoplasm genetic differentiation in the olive complex (*Olea europaea* L.). *Theoretical and Applied Genetics*, 105: 139-144.
21. Edwards, P.C., J. Meadows, G.J. Sayej and M. Westaway, 2004. From the PPNA to the PPNB: new views from the southern Levant after excavations at Zahrat adh-Dhra' 2 in Jordan. *Paléorient*, 30: 21-60.
22. Meadows, J., 2004. The earliest farmers? Archaeological research at Pre-pottery Neolithic A sites in Jordan. In *Studies in the History and Archaeology of Jordan VIII: Archaeological and historical perspectives on society, culture and identity*, Al-Khaysheh F., (ed.), Department of Antiquity of Jordan, pp: 119-128.
23. Liphshitz, N., R. Gophna, G. Bonani and A. Feldstein, 1996. Wild olive (*Olea europaea*) stones from a chalcolithic cave at shoham, Israel and their implications. *Tel Aviv*, 23: 135-142.
24. Liphshitz, N. and G. Bonani, 2000. Dimensions of olive (*Olea europaea*) stones as a reliable parameter to distinguish between wild and cultivated varieties: further evidence. *Tel Aviv*, 27: 23-25.
25. van den Brink, E.C.M., D. Lazar, N. Liphshitz and G. Bonani, 2001. Chalcolithic dwelling remains, cup marks and olive (*Olea europaea*) stones at Nevalat. *Isr. Explor. J.*, 51: 36-43.
26. Trujillo, I. and L. Rallo, 1995. Identifying olive cultivars by isozyme analysis. *J. Ame. Soc. Hortic. Sci.*, 120: 318-324.
27. Hatzopoulos, P., G. Banilas, K. Giannoulia, F. Gazis, N. Nikoloudakis, D. Milioni and K. Haralampidis, 2002. Breeding, molecular markers and molecular biology of the olive tree. *Eur. J. Lipid Sci. and Technol.*, 104: 574-586.
28. Vaughan, J.G. and C.A. Geissler, 1999. *The new Oxford book of food plants*. Oxford University Press, pp: 26-27.
29. Roche, H.M., M.J. Gibney, A. Kafatos, A. Zampelas and C.M. Williams, 2000. Beneficial properties of olive oil. *Food Res. Intl.*, 33: 227-231.
30. Dhifi, W., F. Angerosa, A. Serraiocco, I. Oumar, I. Hamrouni and B. Marzouk, 2005. Virgin oil aroma: Characterization of some Tunisian cultivars. *Food Chem.*, 93: 679-701.
31. Chadwick, J., 1958. *The Decipherment of Linear B*. Cambridge University Press.
32. Simandirakis, V. and M. Lykoudi, 2002. *The olive 'kallistephanos'*. Ephesus Publishing.
33. Hoad, T.F., 1991. *The concise Oxford Dictionary of English Etymology*. The Clarendon Press.
34. Liddell, H.G. and R. Scott, 1991. *A Greek-English Lexicon*. Clarendon Press.
35. Tsougarakis, D., 2004. Olive tree and olive oil in Byzantine times. In *Ode to the olive tree*. Academy of Athens, pp: 101-109.
36. Rhizopoulou, S., 2004. Symbolic plants of the Olympic games. *J. Experi. Bot.*, 55: 1601-1606.
37. Goor, A. and M. Nurock, 1968. *The Fruits of the Holy Land*. Israel University Press.
38. Lacarrière, J., 1975. *L' été Grec*. Plon.
39. Lurker, M., 1988. *The gods and symbols of Ancient Egypt*. Thames & Hudson.
40. Pliny, 1967. *Historia Naturalis*. (Vol.VI), in Latin. Jones, W.H.S. (translator), 1967. William Heinemann.

41. Bitonti, M.B., R. Cozza, A. Chiappetta, A. Contento, S. Minelli, M. Ceccarelli, M.T. Gelati, F. Maggini, L. Baldoni and P.G. Cionini, 1999. Amount and organization of the heterochromatin in *Olea europaea* and related species. *Heredity*, 83: 188-95.
42. Wheeler, G., 1682. A journey into Greece. William Cademan, Robert Kettlewell & Awnsham Churchill.
43. Spon, J., 1724. Voyage d' Italie, de Dalmatie, de Grèce, et du Levant. Rutger Alberts.
44. Wordsworth, C., 1855. Athens and Attica: Notes of a Tour. John Murray.
45. Stearn, W.T., 1976. From Theophrastus and Dioscorides to Sibthorp and Smith: the background and origine of the *Flora Graeca*. *Biolog. J. Linnean Soc.*, 8: 285-298.
46. Lack, W.H. and D.J. Mabberley, 1999. The Flora Graeca Story. Oxford University Press, Oxford.
47. Camp, M.J. and G. Mauzy, 2005. Athenian agora: a guide to the excavation and museum. American School of Classical Studies, Athens.
48. Colonna, F., 1499. Hypnerotomachia Poliphili. The strife of love in a dream. Godwin J, (translator), 2003. Thames & Hudson.
49. Powell, E., 2003. The letters of Vincent van Gogh to his brother and others, Constable and Robinson, 1872-1890.
50. Moreno, F., J.E. Fernandez, B.E. Clothier and S.R. Green, 1996. Transpiration and root water uptake by olive trees. *Plant and Soil*, 184: 85-96.
51. Gucci, R., L. Lombardini and M. Tattini, 1997. Analysis of leaf water relations in leaves of two olives (*Olea europaea*) cultivars differing in tolerance to salinity. *Tree Physiol.*, 17: 13-21.
52. Diamantoglou, S. and K. Mitrakos, 1981. Leaf longevity in Mediterranean evergreen sclerophylls. In *Components of productivity in Mediterranean climate regions basic and applied aspects*, Margaris N.S. and H. Mooney (Eds.), Dr. W. Junk Publishers, pp: 21-27.
53. Benavente-García, O., J. Castillo, J. Lorente and M. Alcaraz, 2002. Radioprotective effects *In vivo* phenolics extracted from *Olea europaea* L. leaves against X-ray chromosomal damage: comparative study versus several flavonoids and sulfur-containing compounds. *J. Med. Food*, 5: 125-135.
54. Rhizopoulou, S., M.S. Meletiou-Christou and S. Diamantoglou, 1991. Water relations for sun and shade leaves of four Mediterranean evergreen sclerophylls. *J. Experi. Bot.*, 42: 627-635.
55. Giorio, P., G. Sorrentino and R. d' Andria, 1999. Stomatal behaviour, leaf water status and photosynthetic response in field-grown olive trees under water deficit. *Environm. Exper. Bot.*, 42: 95-104.
56. Bacelar, E.A., C.M. Correia, J.M. Moutinho-Pereira, B.C. Gonçalves, J.I. Lopesm and J.M.G. Torres-Pereira, 2004. Sclerophylly and leaf anatomical traits of five field-grown olive cultivars growing under drought conditions. *Tree Physiol.*, 24: 233-239.
57. Fahn, A., 1986. Structural and functional properties of trichomes of xeromorphic leaves. *Ann. Bot.*, pp: 631-638.
58. Rhizopoulou, S. and K. Mitrakos, 1990. Water relations of evergreen sclerophylls. I. Seasonal changes in the water relations of eleven species from the same environment. *Ann. Bot.*, 65: 171-178.
59. Karabourniotis, G., N. Papastergiou, E. Kabanopoulou and C. Fasseas, 1994. Foliar sclereids of *Olea europaea* may function as optical fibres. *Canadian J. Bot.*, 72: 330-336.
60. Proietti, P. and A. Palliotti, 1997. Contribution of the adaxial and abaxial surfaces of olive leaves to photosynthesis. *Photosynthetica*, 33: 63-69.
61. Chartzoulakis, K., A. Patakas and A. Bosabalidis, 1999. Changes in water relations, photosynthesis and leaf anatomy induced by intermittent drought in two olive cultivars. *Environmental Experimental Bot.*, 42: 113-120.
62. Minnocci, A., A. Panicucci, L. Sebastiani, G. Lorenzini and C. Vitagliano, 1999. Physiological and morphological responses of olive plant to ozone exposure during a growing season. *Tree Physiology*, 19: 391-397.
63. Beerling, J. D. and G.W. Chaloner, 1993. Stomatal density responses of Egyptian *Olea europaea* L. leaves to CO₂ change since 1327 BC. *Ann. Bot.*, 71: 431-435.
64. Royer, D.L., 2001. Stomatal density and stomatal index as indicators of paleoatmospheric CO₂ concentration. *Rev. Palaeobotany and Palynol.*, 114: 1-28.
65. Osborne, C.P., I. Chuine, D. Viner and F.I. Woodward, 2000. Olive phenology as a sensitive indicator of future climatic warming in the Mediterranean. *Plant Cell and Environment*, 23: 701-710.
66. Osborne, C.P., P.L. Mitchell, J.E. Sheehy and F.I. Woodward, 2000. Modelling the recent historical impacts of atmospheric CO₂ and climate change on Mediterranean vegetation. *Global Change Biol.*, 6: 445-458.
67. Rhizopoulou, S., 2005. Plants as estimates of civilization: first we take Oedipus then we take Theophrastus. In the *Proceedings of the XVII International Botanical Congress*, pp: 634 (P2516).

Evaluation of a Rice Reaper Used for Rapeseed Harvesting

¹Mohammad Reza Alizadeh, ²Iraj Bagheri and ³Mir Hussein Payman

¹Rice Research Institute of Iran (RRII), Rasht, Iran

²College of Natural Resources, University of Guilan, Sowmea Sara, P.O. Box 1144, Iran

³College of Agriculture, University of Guilan, Rasht, P.O. Box 41335-3179, Iran

Abstract: Introduction of appropriate machinery is one of the major factors for reducing labour requirements and production costs of second crop cultivation after rice especially rapeseed. In this study, performance of power tiller-mounted rice reaper used for rapeseed harvesting was assessed and compared with manual harvesting using sickle. The results showed that the effective field capacity of the reaper was 0.170 ha/h compared to 0.008 ha/h for manual harvesting. Labour requirements for reaper and manual harvesting were 5.88 and 128 man-h/ha, respectively. The grain losses for manual and reaper harvesting were 7.33% and 6.83%, respectively. There were no significant differences between means of losses in the two methods. The cost of harvesting operation (without threshing and handling costs) was 88.88\$/ha for manual harvesting and 15.20\$/ha for reaper harvesting (mechanical harvesting). The break-even point of the machine is 4.83ha/year; therefore if the machine (power tiller and reaper combined) works less than this amount it is not economical and renting machine should be considered.

Key words: Rapeseed % harvesting machinery % manual harvesting % paddy fields

INTRODUCTION

Rapeseed is the third most important oil-producing crop after soybean and palm. It accounts for 14% of vegetable oil produced in the world. The most important rapeseed producing countries are China, Canada, India and some European countries (France, England and Germany), which produce 89.4% of the total production. Rapeseed cultivation area in Iran is 129229 hectares producing 213000 tonnes rapeseed per year [1].

Special qualities of rapeseed plant and its adaptability to weather condition in most part of the country have increased the cultivation area of this crop. One of the areas that have been encouraged to grow rapeseed after rice harvesting is the paddy field of Caspian Sea, but despite efforts made the anticipated target have not been reached. The unsuitable physical condition of paddy fields soil, lack of desirable drainage system, lack of appropriate machinery and implements, small fields and simultaneous rapeseed harvesting and rice cultivation, are some difficulties for the development of rapeseed cultivation in this area. Rapeseed harvesting is one of the

crucial stages with regards to quantity and quality of the produced crop and the production costs is also important. The harvesting time of rapeseed is coincided with the start of rice cultivation (land preparation and rice transplanting) and is faced with lack of labour and high wages which is a major problem. On the other hand low work efficiency with manual harvesting delays harvesting operations of the rice crop. To alleviate the problems concerned with growing rapeseed in the paddy fields after harvesting rice, development of mechanization and introduction of suitable machinery specially harvesting machinery is inevitable.

Combine harvesters are used to harvest rapeseed in most part of the country but are faced with limitation in small paddy fields of Caspian Sea. The small field sizes and low traffic capability of soil to withstand the weight of combine at harvesting, causes an increase in losses and also decrease combine field efficiency and capacity. Then again, because of the weather condition and probable spring rainfall, the increase in soil moisture content and field water clogging make the movement of combine harvester difficult in most fields. Further more the time needed for

the crop to reach the required moisture content that is necessary for combine harvesting causes delay in the rice transplanting operation and therefore leading to a decrease in rice crop yield.

In most rapeseed growing countries, the swather and windrower are also used for harvesting the crops. The suitable combination of harvesting machinery and threshers is dependent on the economic and climate conditions and type of crop variety in each area. There is no doubt that the cost of machinery and the labour requirement for each method of harvesting are also effective factors that determine the choice of harvesting method. Choice of suitable harvesting method not only reduces production costs but also increases yield and quality of oil produced [2].

Becel and Mayko [3] studied the effect of direct harvesting with combine harvester on rapeseed yield in western Canada. They reported that since 1985 most rapeseed producers in western Canadian replaced the two stages harvesting using swather with combine harvester. The advantages of combine harvesting is the elimination of swathing operation and the cost involved with that and also time saving, but the results from their research on harvesting different varieties with combine and swather indicated that the choice of suitable method of harvesting also depend on the crop variety. The combine harvester was most efficient for some varieties, where as swathers were more efficient for other varieties.

In some countries, the rice reaper is used for harvesting other crops such as soybean. In Thailand with some modification on rice reaper such as stronger blades, reducing minimum cutting height from 80mm to 40mm, changing distance between star wheels from 30mm to 40mm and increasing blades stroke speed from 437 to 487 rpm, it was used for harvesting soybean. The tests showed that with these modifications, the amount of harvesting losses decreased from 13.2% to 6.27%. The cutting width of the machine was 1.2 m and was powered with a 5.5 horse power petrol engine. The machine field efficiency was 0.083 ha/h compared to 0.005 ha/h of manual harvesting with sickle. The forward speed of machinery was 2.5 km/h. In northern part of Iran the rice reaper is used only for a month in rice harvesting season and is not used in any other part of the year. The objective of this study was to assess performance, grain losses and operational costs of rice reaper which was used for rapeseed harvesting and compare them with manual harvesting method.

MATERIALS AND METHODS

The experiment was conducted in research farm of the Rice Research Institute of Iran (RRII) near the city of Rasht in 2004. The previous crop was rice and rapeseed was planted after rice being harvested. The planting method of rapeseed in this region is usually by broadcasting and row planting. The rapeseed harvesting was performed manually (with sickle) and mechanically (with reaper). The reaper used for harvesting was mounted on a power tiller of 5 kW (Kubota GA-70); the cutting width of the machine was 1.1m. The weight of reaper and power tiller were 70 and 168 kg, respectively. The cutting height of the reaper can be adjusted from 0.2 to 0.55 m. The parameters that were measured during crop harvesting are as follows:

Speed of travel (forward speed): For measuring forward speed of power tiller while harvesting crop, the distance the tiller traveled in 15 seconds was measured and the speed of travel was recorded in terms of km/h.

Time losses and effective operating time: Time losses while harvesting crop is the time for adjustments, turning, fuelling and etc. The start and finish time of harvesting in each plot was also noted.

Field efficiency: Field efficiency is the ratio of effective operating time to total operating time (the ratio of the time a machine is effectively operating to the total time the machine is committed to the operation), in each plot and was determined by the following equation [4]:

$$e = \frac{T_e}{T_t} \times 100 \quad (1)$$

Where,

e = Field efficiency (%)

T_e = Effective operating time (min)

T_t = Total operating time (min)

Effective field capacity: Effective field capacity is the actual rate of performance of land or crop processing in a given time, based on total field time. In other words effective field capacity of a machine is a function of the rated width of the machine, the percentage of rated width actually utilized, the speed of travel and the amount of field time lost during the operation. In order to determine effective field capacity the rated width of implement

(cutting width), Speed of travel and field efficiency were measured. The effective field capacity was calculated by the following equation [5]:

$$C_e = \frac{SW_e}{10} \quad (2)$$

Where,

C_e = Effective field capacity, in hectares per hour (ha/h)

S = Speed of travel, in kilometres per hour (km/h)

W = Rated width of implement, in meters (m)

e = Field efficiency, in percent (%)

Harvesting losses: In order to estimate harvesting losses in manual and reaper harvesting, first the losses that occur before harvesting (preharvest) must be measured. To do this, in four parts of each plot with the use of a wooden frame with 1m×1m dimensions, all grains fallen within the frame are collected and weighed and the mean of the four measured values are recorded. Harvesting losses include shattering and uncut losses and were determined by the following equation [6]:

$$W_{gt} = W_{g1} + W_{g2} + W_{g3} \quad (3)$$

Where,

W_{gt} = Total losses (g/m²)

W_{g1} = Preharvest losses (g/m²)

W_{g2} = Shattering losses (g/m²)

W_{g3} = Uncut losses (g/m²)

After measuring the amount of losses at different stages, the percentage of harvest losses were determined by the following equation [6]:

$$H = \frac{W_{gt} - W_{g1}}{Y_g} \times 100 \quad (4)$$

Where,

H = Percentage of harvest losses (%)

W_{g1} = Preharvest losses (g/m²)

W_{gt} = Total harvesting losses (g/m²)

T_g = Grain yield (g/m²)

Harvesting costs: In order to compare harvesting costs in manual and reaper methods, all the costs of wages in manual and the fixed and variable costs in mechanical operations were calculated. A fixed cost are depreciation cost, interest, shelter and taxes and is a function of purchase value, useful life and interest rate [7].

Depreciation was determined from straight-line method by the following equation [4]:

$$D = \frac{P - V_s}{L_u} \quad (5)$$

Where,

D = Mean yearly depreciation (\$/y)

P = Purchase value (\$)

V_s = Salvage value (\$)

L_u = Useful life (Y)

Useful life for power tiller and reaper was considered to be 10 and 5 years, respectively. The machine salvage value was considered to be 10% of purchase value [7]. Interest is an actual cost in agricultural machinery and was determined from straight line method by the following equation [4]:

$$I = \frac{(P + V_s)}{2} \times i \quad (6)$$

Where,

I = Mean interest (\$/y)

P = Purchase value (\$)

V_s = Salvage value (\$)

i = Interest rate (%)

The insurance and shelter costs were 25% of purchase value [8]. Variable costs include fuel, lubricant, repair and operators costs and are directly related to the amount of work done by the machine. Repair cost for power tiller and reaper was considered to be 5% of purchase value for every 100 hours of effective operation. [7]. Lubricant cost is 25% of fuel cost. The operator wages was 1.11 \$/h (on the basis of the 2001 wages price list). The wages of labour in manual method of harvesting using sickle was also calculated and it was 5.55 \$/day (eight hours of work per day).

The break-even point, the area that a machine has to work per year in order to justify owning the machinery is determined by the following equation:

$$B_e = \frac{F_c}{V_{ct} - V_m} \quad (7)$$

Where,

B_e = Break-even point (ha/y)

F_c = Fixed costs (\$/y)

V_{ct} = Variable costs for manual method (\$/ha)

V_m = Variable costs for machinery method (\$/ha)

RESULTS AND DISCUSSION

Plant specification: Some of the agronomical specifications measured while harvesting rapeseed are shown in Table 1. Each measurement in the Table 1 is a mean value of 10 measurements that were obtained randomly in each plot. It can be seen that, the first secondary stem is just above the ground, this caused the cutting height of blade to decrease to the minimum possible level of 25 mm. The stem thickness in broadcast and row crop planting was on average 14.2 mm but stem thickness differences were large. So that some of the stem thickness was more than 20 mm and some were less than 7 mm.

Reaper performance: Measures of the reaper performance are the rate and quality at which the operations are accomplished. The mean value of some of the parameters that include time losses; total operating time, cutting width, forward speed, effective field capacity and field efficiency are shown in Table 2. The cutting width was 1.1 meter and the forward speed of the machine was 2.14 and 2.23 km/h for broadcast and row crop planting methods, respectively and the mean forward speed for the two methods was 2.18 km/h. Studies carried out by Alizadeh [9] showed that forward speed of reaper mounted on a power tiller for harvesting rice was 2.41 km/h which is higher than the rapeseed harvester.

The results showed that the machine field efficiency is less than its stated field efficiency that is quoted by the manufacturer. The reason for low field efficiency is small fields and increase in time losses. Field efficiency for broadcast and row planting were 68.7% and 73.7 %, respectively. The field efficiency for rapeseed reaper was

less than rice reaper. The studies by Alizadeh [9] showed that the mean field efficiency for mounted rice reaper was 82% compared to 71% for the same reaper for rapeseed harvesting.

The effective field capacity of the reaper for broadcast and row planting methods was 0.161 and 0.180 ha/h, respectively and there was no significant difference. The effective field capacity of machine is a function of speed of travel, field efficiency and cutting width. In manual harvesting with sickle, a labourer on average can harvest 80 m²/h, but this amount can differ with respect to crop condition, labourer ability and climate condition. The required time for harvesting one hectare of rapeseed in manual harvesting was 128 man-h/ha compared to 5.88man-h/ha for the reaper harvesting (Table 2).

Harvesting losses: The measured values of preharvest and harvesting losses in manual and reaper methods are shown in Table 3. The results revealed that the preharvest losses were considerably high and that harvesting was carried out at lower moisture content than normal limit. Delay in harvesting caused grains to shatter due to natural factors (rain and wind) and therefore losses increase. Measurements showed that the moisture content at harvesting time was between 15-18 % and this is not suitable for indirect harvesting (reaping and threshing). If harvesting is carried out at suggested moisture content (25%-30%), the amount of preharvest losses and cutting and handling losses are significantly reduced. Therefore it is necessary to assess the most suitable moisture content for harvesting and its relation to the amount of losses.

Table 1: Some of the estimated agronomic specification of rapeseed in manual and reaper harvesting

Planting method	Height of Main stem (mm)	Height of First secondary stem from ground (mm)	Number of sub-main stems	Plant density (Numbers/m ²)	Thickness of main stem (mm)
Broadcast	1022	29.7	4.4	113.5	13.8
In row	1044	31.3	4.6	102.7	14.6

Table 2: Mean values for the manual and mechanical methods of rapeseed harvesting

Harvesting method	Planting method	Time losses (min)	Total operating time (min)	Cutting width (m)	Forward speed (km/h)	Field efficiency (%)	Effective field capacity (ha/h)
Reaper	Broadcast	3.75	13.0	1.1	2.14	68.7	0.1610
Reaper	In row	3.34	10.6	1.1	2.23	73.7	0.1800
Manual	Broadcast	6.00	55.0	na	na	na	0.0074
Manual	In row	5.00	51.0	na	na	na	0.0086

na = not available

Table 3: Estimated losses in two manual and reaper methods of harvesting

Re P.	Manual planting (broadcasting)						Row planting					
	Harvesting with reaper			Manual harvesting			Harvesting with reaper			Manual harvesting		
	W_{g1}	W_{g2+3}	W_{gt}	W_{g1}	W_{g2+3}	W_{gt}	W_{g1}	W_{g2+3}	W_{gt}	W_{g1}	W_{g2+3}	W_{gt}
1	1.67	8.93	10.60	1.35	7.82	9.17	1.27	7.63	8.90	1.17	6.93	8.10
2	0.97	8.38	9.35	1.76	8.35	10.11	1.53	8.32	9.82	1.47	7.67	9.14
3	1.43	7.72	9.15	1.51	6.87	8.38	1.77	7.52	9.29	1.86	8.16	10.02
4	1.82	9.26	11.08	1.47	8.73	10.20	1.08	8.83	9.91	1.62	7.40	9.07
Ave.	1.47	8.57	10.04	1.52	7.94	9.46	1.41	8.07	9.48	1.53	7.55	9.08

Table 4: Percentage^a of preharvest and harvesting losses for manual and reaper harvesting in manual planting (broadcasting)

Re P.	Manual harvesting			Reaper harvesting		
	$W_{g1}(\%)$	$W_{g2+3}(\%)$	$W_{gt}(\%)$	$W_{g1}(\%)$	$W_{g2+3}(\%)$	$W_{gt}(\%)$
1	0.99	5.98	6.98	0.84	5.00	5.84
2	1.24	6.76	7.98	1.15	6.04	7.20
3	1.32	5.62	6.94	1.37	6.02	7.40
4	0.89	8.16	1.30	6.00	7.32	7.27
Ave.	6.93	5.77	1.16	7.51	6.40	1.11

* Average of four measurements

Table 5: Percentage^a of preharvest and harvesting losses for manual and reaper methods in row planting

Re P.	Manual harvesting			Reaper harvesting		
	$W_{g1}(\%)$	$W_{g2+3}(\%)$	$W_{gt}(\%)$	$W_{g1}(\%)$	$W_{g2+3}(\%)$	$W_{gt}(\%)$
1	1.21	6.50	7.72	0.91	5.27	6.18
2	0.71	6.20	6.91	1.31	6.25	7.56
3	1.00	5.42	6.42	1.00	4.51	5.51
4	1.26	6.40	7.66	1.11	6.62	7.73
Ave.	1.04	6.12	7.16	1.08	5.66	6.74

* Average of four measurements

Table 6: Calculation for costs of the reaper machine in rapeseed harvesting

Case	Power tiller*	Reaper	Total
Purchase value(\$)	1000	611.11	1611.11
Machine life (year)	10	5	-
Annual use (hours)	700	480	-
Salvage value (\$)	100	61.11	-
Fixed costs			
Depreciation (\$/h)	0.128	0.229	0.357
Interest (\$/h)	0.125	0.112	0.237
Shelter and insurance (\$/h)	0.028	-	0.028
Total fixed costs (\$/h)	0.281	0.341	0.622
Variable costs			
Labourer	1.11	-	1.11
Fuel	0.035	-	0.035
Oil	0.008	-	0.008
Repairs	0.500	0.305	0.805
Total variable costs	1.653	0.305	1.958
Effective field Capacity (ha/h)	-	0.170	-
Harvesting time (h/ha)	-	5.88	-

* Power tiller with necessary equipments

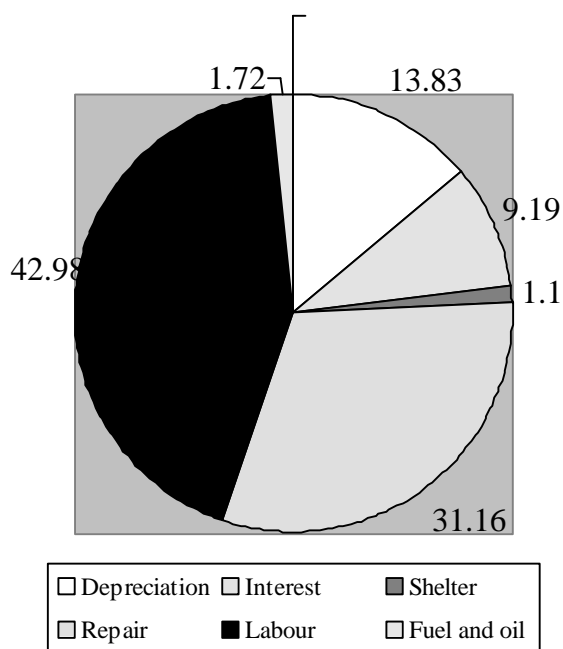


Fig. 1: Comparison of fixed and variable costs of the reaper

The percentage of preharvest, harvesting and total harvesting (preharvest and harvesting) losses in broadcast and row planting are shown in Tables 4 and 5, respectively. The mean preharvest losses in manual and reaper harvesting were 1.07% and 1.12% respectively and there is no significant difference between them. The harvesting losses in manual and reaper harvesting were 6.26% and 5.71% respectively. The total harvesting losses in manual and reaper harvesting were 7.33% and 6.83%. There was no significant difference in harvesting losses in the two broadcasting and row planting method.

Harvesting costs: The fixed and variable costs for harvesting rapeseed with reaper are shown in Table 6. The comparison of fixed and variable costs per hectare are shown in Fig. 1. The fixed cost accounts for 24% of machine cost and the reason for this is high purchase value of the reaper and power tiller. Also due to high interest rate, the interest cost is a major part of fixed cost. Repair and labour costs account for 31% and 43% of total machine cost, respectively. The high cost of spares and repair rate increases repair cost. Lack of authorized repair shops and suitable after sale services are also a reason for high repair rate and spare costs (Fig. 1). Labour requirement for reaper harvesting was 5.88 man-h/ha compared to 128 man-h/ha for manual harvesting. The cost of harvesting operation (without threshing and handling costs) in manual method was 88.88 \$/ha and that of reaper harvesting was 15.20 \$/ha.

The break-even point of the machine was 4.83 ha/year; therefore if the machine (power tiller and reaper combined) works less than this amount then it is not economical and renting machine should be considered. The cost of renting a reaper is 11.10 \$/h and the time needed for harvesting is 5.88 h/ha; therefore the cost of renting reaper is 65.27 \$/ha and comparing it with manual method there is about 27% reduction in cost.

CONCLUSION

From the analysis of the results the following can be concluded:

- C The effective field capacity of the reaper for rapeseed harvesting was 0.170 ha/h compared to 8×10^3 ha/h in manual operation.
- C The average labour requirements for reaper and manual harvesting were 5.88 and 128 man-h/ha, respectively. Therefore in fields where the use of reaper is possible, it will play an important role in reducing production costs.
- C The average grain losses for reaper harvesting were 6.83% compared to 7.33% in manual method. In two stages harvesting of rapeseed with reaper, assessment of the most suitable moisture content at harvesting time is necessary in order to reduce percentage of losses.

- C The cost of harvesting operation (without threshing and handling costs) in manual method was 88.88 \$/ha and in reaper harvesting was 15.20 \$/ha; a reduction of about 83% in harvesting cost. Fixed cost is a major part of total machine operation, bank loan facilities with low interest rate and long repayment time can effectively reduce this cost.
- C For economical justification of machine application, the yearly capacity of machine must not be less than about 5ha. Therefore facilities should be given to farmers to increase cultivation area especially where the lands have been consolidated, which can increase machine field efficiency.
- C In order to increase the reaper performance, studies should be carried out on the machine working parameters and the appropriate rapeseed crop conditions in harvesting operation.

REFERENCES

1. Statistics Bureau, 2005. Statistics Bureau, Ministry of Jihad and Agriculture Statistics, Iran.
2. Ahmadi, M., 2000..Method and time of harvesting rapeseed. Extension Journal, Institute of research, education and agricultural extension, Extension department, Iran, pp: 24.
3. Becel, J.H. and J.J. Mayko, 1995. Direct combining of canola in western Canada, Canada.
4. Kepner, R.A., R. Bainer and E.L. Barger, 1982. Principle of Farm Machinery.3rd edition, avi, West port,Connecticut.
5. Bukhari, S.B., J.M. Baloch and A.N. Mirani, 1989. Soil manipulation with tillage implements. AMA, 20: 17-20.
6. Pradhan, S.C., R. Biswajit, D.K. Das and M. Mahapatra, 1998. Evaluation of various paddy harvesting methods in Orissa. India. AMA, 20: 35-38.
7. Singh, G., A.P. Chaudhary and D.S. Clough, 1988. Performance evaluation of mechanical reapers in Pakistan. AMA, 19: 47-52.
8. Kathirvel, K., T.V. Job, R. Karunanithi and K.R. Swaminathan, 1990. Development of auger digger as attachment to power tiller. AMA., 21: 9-10.
9. Alizadeh, M.R., 2002. Field efficiency evaluation of reaper machinery harvesting rice and comparing it with traditional method. Journal of Agricultural Machinery Research, Iran, 3: 1-14.

Cytotoxic Activity of *Amorphophallus paeoniifolius* Tuber Extracts *In vitro*

J. Angayarkanni, K.M. Ramkumar, T. Poornima and U. Priyadarshini

Department of Biotechnology, Bharathiar University, Coimbatore, 641 046, Tamilnadu, India

Abstract: *Amorphophallus paeoniifolius*, belongs to the family Araceae, widely distributed in tropical and subtropical regions and are extensively used in South India for various diseases. The present investigation evaluates the cytotoxic property of the different solvent extracts of *A. paeoniifolius* tuber using *Allium cepa* L. root tip cells and HEP-2 cell line as two model *in vitro* systems. Of the seven different extracts of *Amorphophallus* tuber were tested, the mitotic index and cytolytic index were found to be high in petroleum ether and ethanol fractions when compared with other solvent extracts. The magnitude of cytotoxicity was predominant in petroleum ether extract and ethanolic extract and displayed a dose dependent antiproliferative activity on HEP2 cells. Present study thus confirms the cytotoxic property of *A. paeoniifolius* and also demonstrated the role of *A. paeoniifolius* used in the traditional medicine.

Key words: *Amorphophallus paeoniifolius* % medicinal plants % antiproliferative activity % *Allium cepa* L. root tip cells % HEP-2 cell line

INTRODUCTION

In recent years the popularity of complementary medicine has increased. Over 50% of all modern clinical drugs are natural product origin and they play an important role in drug development programs of the pharmaceutical industry [1]. Epidemiological evidence suggests that dietary factors play an important role in human health and in the treatment of certain chronic diseases including cancer [2, 3]. Some dietary sources contain antitumor compounds [4] and such compounds are candidates for chemo preventive agents against cancer development [5]. The anticancer property of nutrients derived from plants as well as nonnutritive plant derived constituents has been proved in different *in vitro* and *in vivo* models [6], which had led to an increased emphasis on cancer prevention strategies in which these dietary factors are utilized [7]. Dietary measures and traditional plant therapies as prescribed by ayurvedic and other indigenous systems of medicine are used commonly in India [8].

Amorphophallus paeoniifolius, (Araceae) is a commonly available tuber in South India, widely used in folk medicine for acute rheumatism, tumors, lung swelling, asthma, vomiting and abdominal pain. It is also a major ingredient of several Indian herbal prescriptions. So far, no attempts have been made to evaluate the medicinal properties of *A. paeoniifolius*. Hence the present study

was performed to investigate the cytotoxic effects of different solvent extracts of *A. paeoniifolius* using *Allium cepa* L. root tip cells as well as HEP-2 cell line (a human larynx epithelial carcinoma cell line).

MATERIALS AND METHODS

Plant material: *Amorphophallus* tuber was collected from Coimbatore, Tamilnadu. The tuber was identified with the Herbarium of Botanical Survey of India, Southern Circle, Coimbatore, as *Amorphophallus paeoniifolius* and was deposited in the Department of Biotechnology, Bharathiar University.

Tuber extract preparation: The tuber of the plant was dried under shade and made to a fine powder (particle size ~0.25mm) using a laboratory mill and was extracted subsequently with a series of organic solvent with increasing polarity by using soxhlet extractor. The order of extraction was petroleum ether, benzene, chloroform, ethyl-acetate, acetone, ethanol and methanol. The isolated fractions were weighed and yield was calculated. Further, the extracted fractions were analyzed for its antiproliferative properties.

Plant cytotoxicity analysis: The extracts (1% w/v, in DMSO) of *A. paeoniifolius* tuber were subjected to plant cytotoxicity analysis. For this study *Allium cepa* L. root

tip cells were used. Onions were placed with aerated water at room temperature to root for 24 h. DMSO was used as control. The control group was considered as time zero (0-h) until the first root sample was obtained. This root sample was then placed for 24h in extracts of the *A. paeoniifolius* tuber. After this time period a few root tips were removed and the bulbs were returned to water, for further 24h, to observe if there was recovery from possible damage. The treated roots were fixed and stained by acetocarmine and mounted on permanent slides. The slides were analyzed under microscope with 40X objective lens. Cells were examined for morphological and structural alterations and the mitotic index and cytolytic index were determined [9].

Animal cell cytotoxic assay: Cytotoxic assay was performed as described by Tengchaisri *et al.* [10] using HEP-2 cell line. Briefly, HEP-2 cells suspended in Minimal essential medium (MEM) containing 10% FBS were seeded at 1×10^4 cells (100 μ l) per well in 96-well plate and incubated in humidified atmosphere with 95% and 5% CO₂ at 37°C. After 24 h, additional medium (100 μ l) containing the test compound (different concentrations) dissolved in 0.2% DMSO was added and further incubated for 24 h. The viability in cultured cells was determined by trypan blue exclusion assay. Cells were harvested using 0.025% trypsin, incubated with 4% trypan blue solution and were counted using a hemocytometer under light microscope. Cells failing to exclude the dye were considered non viable and the number of nonviable cell was expressed as a percentage of the total cells.

RESULTS

In the present study, *A. paeoniifolius* tuber was extracted with organic solvent. The extracts were analyzed for its antiproliferative properties using *Allium cepa* L. root tip cells and HEP-2 cells.

Tuber of *A. paeoniifolius* was extracted subsequently with petroleum ether, benzene, chloroform, ethyl acetate, acetone, ethanol and methanol. The percentage yield of this extraction ranged between 1 to 7% (Fig. 1). The highest yield of 6% and 6.7% were observed in case of petroleum ether extraction and ethanol extraction.

All the seven extracts of *Amorphophallus* tuber were subjected to plant cytotoxicity analysis using *Allium cepa* L. root tip cells. Only petroleum ether and ethanol showed low mitotic index of 0.34% and whereas the cytolytic index were found to be 75% for petroleum ether and 80% for ethanol extract. The mitotic index and cytolytic index were

Table 1: Plant cytotoxicity analysis in *Allium Cepa* L. root tip of different extracts of *A. paeoniifolius*

Treatments	Total number of cells	Mitotic index (%)	Cytolytic index (%)
Control (DMSO)	148	11.90	1.2
Petroleum ether	100	0.34	75.0
Benzene	85	3.50	15.0
Chloroform	82	1.40	9.0
Acetone.	150	5.00	4.8
Ethyl acetate	148	2.40	5.9
Ethanol	145	0.34	80.0
Methanol	105	6.22	6.0

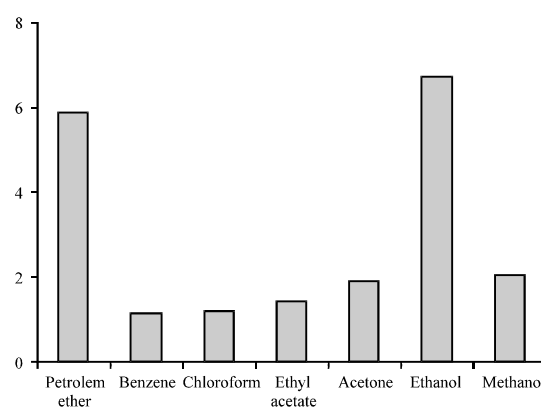


Fig. 1: Percentage yield of constituents in different extraction of *A. paeoniifolius*

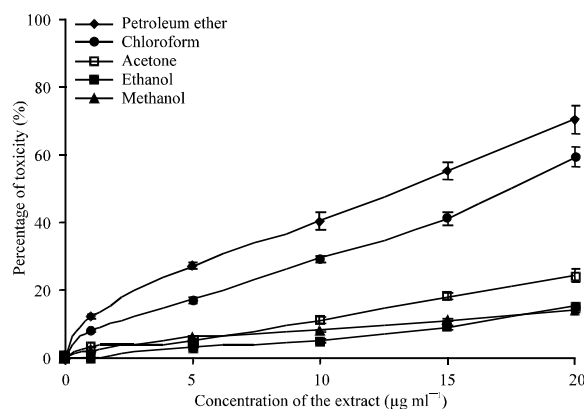


Fig. 2: Cytotoxicity of different extracts of *A. paeoniifolius* on HEP2 Cell lin

found to be low in chloroform, acetone and methanol. The petroleum ether and ethanol were found to be highly antiproliferative property (Table 1).

Of the seven different extracts of *Amorphophallus* tuber were tested, only ethanol and petroleum ether extract displayed a dose dependent antiproliferative activity on HEP₂ cells (Fig. 2). In the present study, HEP₂ epidermoid cell line was used which is the best model to

study the cytotoxicity assay. Untreated Hep-2 cells appeared as elongated shape, attached smoothly on the culture surface and some of the cells grouped together to form colonies. Following treatments with extract for 24 hrs, the cells changed to round shape and lost cell contacts. In particular, the cells lost their surface morphology and died at a concentration of 60 and 70%. Further the viability assay using trypan blue dye showed the maximum number of death percentage in petroleum ether extract when compared to ethanol extract which showed the secondary level of death of cells. Whereas the magnitude of cytotoxicity was predominant in petroleum ether extract and ethanolic extract when compared to other extracts. Since the cytotoxicity was not determined in ethyl acetate and benzene extract (Data not given), however very less antiproliferative activity was observed in chloroform and acetone extract.

DISCUSSION

Plant substances continue to serve as viable source of drugs for the world population and several plant-based drugs are in extensive clinical use [11]. Agents capable of inhibiting cell proliferation, inducing apoptosis or modulating signal transduction are currently used for the treatment of cancer [12]. The use of multiple chemopreventive agents or agents with multiple targets on cancer cells are considered to be more effective in cancer treatment [13].

An assessment of their cytotoxic and mutagenic potential is necessary to ensure antiproliferative property. The ethanol extract showed low mitotic index of 0.34% and whereas the cytolytic index were found to be 80% for ethanol. Teixeira *et al.* [14] have reported that infusions prepared from the medicinal plants *Psidium guajava* L. and *Achillea millefolium* L. showed mitotic index of 1.1% and no activity for *Achillea millefolium* L. which comparatively less than the present investigation. The cell growth recovered in both the plants after 24 hrs treatment whereas there is no recovery in the present analysis.

Recent reports have cited that so many plants and its components could act as tumor suppressor, apoptotic inducers in cancer cells. For example Ginseng from *panax ginseng*, the most commonly used herbal medicine have tumor suppressing activity, interfere with cell cycle progression, enhance immune activity and suppress tumor angiogenesis [15]. Likewise the aqueous extract of *Helixanthera parasitica* is also reported [16]. In the present study the *Amorphophallus* tuber extracts is well correlated with previous reports from different

plant extracts on cancer suppressing activity or anticarcinogenic activity.

In conclusion, the results of these investigations should be helpful in the better explaining the complex pharmacological activity of *Amorphophallus* tuber. Following these studies the present study confirms the potential of *A. paeoniifolius* extracts can be used as anticancer drug. Further more mechanistic work is essential to prove these compounds as a one of the specific cancer drug.

REFERENCES

1. Baker, J.T., R.P. Borris, B. Carte, G.A. Cordell and D.D. Soejarto *et al.*, 1995. Natural product drug discovery and development: New perspective on international collaboration. *J. Natl. Prod.*, 58: 1325-1357.
2. Trichopoulos, D. and W.C. Willett., 1996. Nutrition and cancer. *Cancer Causes Cont.*, 7: 3-4.
3. Block, G., 1992. The data support a role for antioxidants in reducing cancer risk. *Nutr. Rev.*, 50: 207-213.
4. Rogers, A.E., S.H. Zeisel and J. Groopman, 1993. Diet and carcinogenesis. *Carcinogenesis*, 14: 2205-2217.
5. Dorai, T. and B.B. Aggarwal, 2004. Role of chemo preventive agents in cancer therapy. *Cancer Lett.*, 215: 129-140.
6. Barnes, S., 1995. Effect of Genistein on *in vitro* and *in vivo* Models of Cancer. *J. Nutr.*, 125: 777-783.
7. Singh, B., T.K. Bhat and B. Singh, 2003. Potential therapeutic applications of some antinutritional plant secondary metabolites. *J. Agric. Food. Chem.*, 51: 5579-5597.
8. Agrawal, D.P., 2002. Complementary and alternative medicine: an overview. *Curr. Sci.*, 82: 518-524.
9. Knoll, M.F., A.C.F. Da Silva, T.S.D. Canto-Dorow and S.B. Tedesco, 2006. Effects of *Pterocaulon polystachyum* DC. (Asteraceae) on onion (*Allium cepa*) root-tip cells. *Genet. Mol. Biol.*, 29: 539-542.
10. Tengchaisri, T., R. Chawengkirtikul, N. Rachaphaew, V. Reutrakul, R. Sangsuwan and S. Sirisinha, 1998. Antitumor activity of TPL against cholangiocarcinoma growth *in vitro* and in hamsters. *Cancer Lett.*, 133: 169-175.
11. Heinrich, M. and P. Bremner, 2006. Ethnobotany and ethnopharmacy-their role for anti-cancer drug development. *Curr. Drug Targets*, 7: 239-245.

12. De Flora, S. and L.R. Ferguson, 2005. Overview of mechanisms of cancer chemopreventive agents. *Mutat. Res.*, 591: 8-15.
13. Howells, L.M. and M.M. Manson, 2005. Prospects for plant-derived chemopreventive agents exhibiting multiple mechanisms of action. *Curr. Med. Chem. Anticancer Agents*, 5: 201-213.
14. Teixeira, R.D.O., L.C. Marjori, M.S. Mantovani and V.E.P. Vicentini, 2003. Assessment of two medicinal plants, *Psidium guajava* L. and *Achillea millefolium* L., in *in vitro* and *in vivo* assays. *Gent. Mol. Biol.*, 26: 551-555.
15. Sato, K., M. Mochizuki, I. Saiki, Y.C. Yoo, K. Samukawa and I. Azuma, 1994. Inhibition of tumor angiogenesis and metastasis by a saponin of *Panax ginseng*, ginsenoside-Rb2. *Biol. Pharm. Bull.*, 17: 635-639.
16. Lirdprapamongkol, K., C. Mahidol, S. Thongnest, H. Prawat *et al.*, 2003. Anti-metastatic effects of aqueous extract of *Helixanthera parasitica*. *J. Ethnopharmacol.*, 86: 253-256.

The Development of Production, Export and Domestic Sales of Organic Agricultural Products in Turkey

Osman Kızılcı and İsmail Alkan

Department of Agricultural Economics, Faculty of Agriculture,
Ondokuz Mayıs University, 55139, Samsun - Turkey

Abstract: Sensitivity concerning environmental protection and demand for healthy food have been showing an escalating trend foremost in developed countries in recent years. For this purpose, production of organic agricultural products has become important in many countries around the world. The USA and EU countries are the leading countries which are attaching great importance to organic food production and consumption. Increase in the demand for organic products has created a need for cheaper and better quality organic production. The production of organic agricultural products has now created an additional export opportunity for developing countries such as Turkey. Despite having suitable conditions for organic production, Turkey's share of world organic production and marketing is very low. Turkey's share is only 0.1% in the world organic market of \$20 billion. Unlike the developments in Europe, organic agriculture activities in Turkey have begun to incline in accordance with the demand of importer companies. In the last decade in Turkey, legislation pertaining to organic agricultural products has come into force. The number of organic products had risen to 174 in 2004 from 8 in 1990. The reasons for this development are the education of farmers and technicians, producer organizations and important enterprises in marketing.

Key words: Organic agriculture % organic production % organic agriculture legislation % sustainable agriculture % Turkey

INTRODUCTION

As known, the propensity to consume escalates if population and income increase. Because of this fact, the world demand for food is increasing daily. To meet need for food, agricultural production per capita should be increased. The most effective way of increasing agricultural production is raising the yield. Raising the yield is a consequence of more intensive input usage. Especially after the Industrial Revolution, chemical input use in agricultural production has shown a marked rise in western countries. However, widespread chemical input use has increased environmental pollution and consequently, food safety has deteriorated. Accordingly, damage to the balance of nature and human health due to the use of excessive chemical input has become better understood. Consequently, searches for alternative approaches began as some developed countries began to restrict the usage of chemical inputs.

Restriction of chemical input use also provided a starting point for organic farming. For this purpose the International Federation of Organic Agriculture Movements (IFOAM) was founded in 1972 [1]. This foundation gathered the world's organic farming organizations under the same umbrella. The establishment of IFOAM and the increase in consumer demand in developed countries for healthier and better quality products lead the way to a rapid increase in the number of organic producers and organic production.

Trade of organic agricultural products began to increase globally after 1980. Today, organic production is practised in approximately 110 countries and on 26 million hectares (ha) area in the world [2]. Organic farming began through contractual farming in 1980's with very few products in Turkey [3]. In 1992, the Ecological Agriculture Organization (ETO) was established in Izmir. An important effect of the establishment of ETO was that the number of organic products increased and production became widespread in the country after 1992.

In Turkey, the most important problem is experienced between exporter companies and producers of organic agricultural products. However, inadequate domestic demand is a serious handicap to the development of organic farming.

MATERIALS AND METHODS

Data for this study were obtained from the Ministry of Agriculture and Rural Affairs, Turkey (MARA), IFOAM (Soel) and Undersecretariat of the Prime Ministry of Foreign Trade/ Export Promotion Center, Turkey (IGEME). Moreover, related literature was also utilized. Statistical methods, such as percentages and averages have been used in the study.

Developments in the production of organic agricultural products in Turkey: Especially after the Second World War, both in developed and to some extent developing countries, agriculture became highly mechanized and specialized as well as heavily dependent on agro-chemicals. Such intensification of farming has produced higher yields and greater wealth but has also created some problems affecting the environment, food and farm-worker safety [4]. As a consequence, organic agriculture became increasingly important. In 1972, the International Federation of Organic Agriculture Movements (IFOAM) was established. The main office of the organization is in Germany and it has more than 750 members in 108 countries [1]. After 1980's, organic agriculture have become a contemporary production system together with the development of alternative production methods. Also, consumer consciousness has had an important role in the development of organic agricultural production.

Today, organic agriculture is practised in approximately 100 countries and more than 26 million ha in the world. The ten countries having the largest organic agricultural area are Australia (11.300.000 ha), Argentina (2.800.000 ha), Italy (1.052.002 ha), USA (930.810 ha), Brazil (803.180 ha), Uruguay (760.000 ha), Germany (734.027 ha), Spain (725.254 ha), UK (695.619 ha) and Chile (646.150 ha) respectively. Turkey is twenty ninth ranked on the list with 103.190 ha [2].

When the land area under organic management as a percentage of total agricultural area is analyzed, the five highest ranked countries are Liechtenstein (26.4%), Austria (12.9%), Switzerland (10.3%), Finland (7.2%) and Italy (6.9%) [2].

The number of farms worldwide practising organic agriculture production is approximately 558.500. The top three countries are; Mexico (120.000), Indonesia (45.000) and Italy (44.043). Turkey is twelfth ranked with 13.044 farms [2].

Italy is the top country in Europe in terms of organic agricultural area. Germany, UK, Spain and France follow Italy respectively. Although Sweden, Austria, Denmark, Finland and Switzerland have small organic agricultural areas, they are among the developed countries in the organic agricultural sector [5].

Organic agricultural activities in Turkey became export-oriented mainly after 1986. This was in accordance with the demands of importer companies. In the beginning, production and export was practised according to the legislation of importer countries. After 1991, production and export were carried out in accordance with the European Council Regulation numbered 2092/91. Subsequently, the obligations for countries exporting organic products to the European Union were stated in detail in the appendix numbered 94/92, published in January 14, 1992. Under this arrangement, every country has to prepare its own legislation and apply to the European Union in relation to various technical and administrative subjects and the legislation [6].

In 1992, ETO was founded in order to provide the rapid and stable development of organic agriculture under a specific umbrella organization with the participation of producers, consumers, handlers, auditors, researchers propounding ecological agriculture philosophy. The center for ETO is in Izmir. The main purposes of ETO are to develop and widen organic agriculture and to create a domestic market [7].

The Ministry of Agriculture and Rural Affairs (MARA) brought "The Legislation Concerning the Production of Vegetable and Animal Products by Ecologic Methods" into force in 1994 to accommodate developments in the EU.

In 2004, "Organic Farming Law" which can be considered as a revolution after dense studies, came into force so as to practise organic agriculture extensive [8].

Production of organic agricultural products began in 1983-1984 in Turkey [9]. Organic production began with sultanas and figs which are traditional export products from the Aegean region. Later, hazelnuts and apricots were added to these products [3]. Today, 7 companies are charged with certification and control by MARA in Turkey. Five of them are foreign and two of them are domestic. Furthermore, the number of companies

Table 1: Changes in the number of organic products, organic producers and organic production area in Turkey *[10],**[6]

Years	Number of Products	Number of Producers	Production Area (ha)
1990*	8	313	1037
1992*	23	1780	6077
1994*	20	1690	5196
1996*	37	4039	16000
1998*	65	8302	25303
1999*	92	12435	44552
2000**	95	18385	59985
2001**	98	15795	111324
2002**	147	12428	89826
2003**	176	13044	103190
2004**	174	9314	162193

processing, storing, packing, transporting and marketing organic products is 236 and all of these are registered by MARA. [6].

As seen in Table 1, there has been a steady increase in organic agriculture in terms of products, producers and area in Turkey from the beginning in 1990. While the number of organic products was 8 in 1990, it rose to 174 in 2004. The number of producers increased 30 fold and the production area increased 156 fold between the same years. In 2004, 9.314 producers were practising organic agriculture on an area of 162.193 ha. In 2004, organic agriculture area per producer was 17.4 ha.

Fruits comprise the major part of organic production (66%) in Turkey. Others are field crops (16%), vegetables (9%) and minor products (9%). Especially grapes, figs, apricots and hazelnuts have important production volumes [3].

All of the products produced organically are listed in Table 2. When Table 2 is analyzed, it can be seen that organic agriculture is practised in nearly all agricultural products. The largest organic product ranges are in fruits and vegetables.

The price of an organic product is generally determined by the “market price+premium approach” in Turkey. Price is determined usually at harvest time or at the beginning of the purchase and sale period. Organic product prices are generally 10-15 % more than conventional product prices [7].

Domestic sales of organic agricultural products in Turkey and developments in export: The nurturing of a domestic market is an important alternative solution for the healthy development of organic agriculture. However, lack of demand cannot be disregarded despite substantial

Table 2: Organic agricultural products which are produced commercially in Turkey [11]

Crops	
Hard-shelled fruits	Hazelnuts, Walnuts, Pistachios, Almonds, Peanuts, Chestnuts
Dried fruits	Grape, Apricot, Sliced Apricots, Apricot, Plum, Fig, Apple, Cherry, Sour Cherry, Pear, Strawberry
Dried vegetables	Tomato, Mushroom
Fresh fruits and Vegetables	Apple, Figs, Strawberry, Plum, Pear, Sour Cherry, Cherry, Persimmon, Berry, Watermelon, Lemon, Orange, Grapefruit, Mandarin, Peach, Grape, Tomato, Pepper, Cucumber, Spinach, Leek, Cauliflower, Aubergine, Parsley, Carrot, Potato, Onion, Garlic, Celery, Old buffer, Pea
Pulses	Lentil, Chickpea, Haricot bean
Spices and medical-Perfumed plants	Bay, Thyme, Cumin, Sage, Rosemary, Linden, Fennel, Peppermint, Nettle
Industrial plants	Cotton, Poppy seed, Anise, Sugar beet
Oil seeds	Sunflower, Sesame
Cereals	Wheat, Rice, Corn, Oats, Barley
Others	Capers, Pine nut, Olive, Rose hip, Red Pepper, Hazelnut flour
Processed food products	
Frozen fruits	Apricot, Strawberry, Cherry, Sour cherry, Berries,
And vegetables	Plum, Onion, Courgette, Tomato, Pepper
Fruit juices	Apricot puree, Pear juice concentrate, Sour
And concentrates	cherry concentrate, Apple juice concentrate
Others	Olive oil, Boiled and pounded wheat, Pectin, Tomato puree, Jam, Wine, Embered pepper
Other agricultural Crops	Honey, Apricot kernel, Sour cherry kernel, Dried Rose, Rose Oil, Rose water, Mersin oil, Mersin water, Thyme oil, Lavender oil

effort. The opening of a few shops selling organic products in big cities and establishment of special stands in some supermarkets are positive developments, but on the other hand, it can be said that a domestic market is still not present [12]. Due to the demand deficiency, unconscionness of consumers, lack of promotion, expensiveness of organic products and marketing problems, domestic market is limited in Turkey [3].

In Turkey, while sultana and fig were the only commercial organic agricultural products in 1985, the product range had expanded by 2001. Organic products produced and exported in important volumes were hard-shelled and dry fruits, frozen fruits and vegetables, damp fruits and vegetables, spices and pulses. Rose water, rose oil, olive oil and cotton were other products produced and exported.

Table 3: Organic product export values of Turkey according to countries (in tonnes) [6]

Years	Germany	UK	Netherlands	Switzerland	Italy	Others	Total
1998	3610	593	1222	1400	29	1762	8616
1999	3841	1447	1959	1354	183	3266	12050
2000	4022	1469	1811	1258	399	4170	13129
2001	6213	1716	1670	1311	905	5741	17556
2002	7629	2023	1517	1223	941	5850	19183
2003	7531	1867	3598	1155	1710	5222	21083
2004	5238	1710	1677	822	1381	5265	16093
2004 (%)	32.6	10.6	10.4	5.1	8.6	32.7	100.0

Turkey exports to a total of 37 countries with the EU countries being the most important export markets. The countries of northern Europe, USA, Canada and Japan are potential markets which draw attention [11].

When we analyse the total export and distribution of organic products according to countries (Table 3), it is seen that Germany is the biggest importer country with a share of 32,6% in 2004. The countries following Germany were respectively the UK, Netherlands, Italy and Switzerland [6].

RESULTS AND DISCUSSION

Organic agriculture is expanding daily in Turkey. However it is underdeveloped when compared with European countries. Agricultural land in Turkey is not very contaminated, so changing over organic agriculture is relatively easier. Furthermore, Turkey has a wealthy flora and in this respect Turkey is well placed for the development of organic agriculture.

To create a supportive environment, government should develop agricultural policies encouraging organic agriculture. For example, certain inputs could be supported and credits with special conditions could be allocated to producers practising organic agriculture.

Currently, the organization of organic producers is inadequate at the local and regional level. Producers should be more organized according to products and location on a local and regional basis.

Organic agriculture is a production system requiring 2 or 3 times more man labor when compared with conventional agriculture [7]. Turkey has high unemployment, a large rural population and one-third of Turkey's population is working in the agriculture sector, for these reasons organic agriculture is favorable for Turkey.

Inputs used in organic production are generally hard to obtain. For example, organic fertilizers in markets are scarce and expensive. Organic fertilizers in Turkey are generally imported from abroad. Therefore, organic fertilizer production should be increased in Turkey. In this way, a reduction in both production costs and prices should occur.

Currently, Turkish farmers have inadequate knowledge about organic agriculture. This education deficiency should be addressed rapidly. Agricultural Provincial Directorates and Agricultural District Directorates have this responsibility. Producers state that they do not meaningfully benefit from current courses. These institutions should inform producers about organic agriculture. For example, they could organize workshops of 1 or 2 days.

It is not generally understood by the growers that organic products have positive effects for human health and for the protection of natural sources. On this account, government and private institutions should explain the contributions of organic products to human health and environment. Organic agriculture potential is not appreciated and fostered enough in Turkey and consequently there is demand deficiency. A change in peoples' inclination to purchase organic products will encourage development of the domestic market for organic products. In this way, some organic production will be redirected to internal demand.

In parallel, book keeping by producers should be encouraged. Keeping books should be supported by the relevant institutions. It would then be easier to conduct research about organic products of economic importance.

Today, countries all over the world are making efforts to increase their organic production, accelerate related studies and develop legislations. Laws now oblige the use of organic products in infant food in USA for children of 0-2 years old and in Germany for children of 2-6 years old. In EU countries similar decisions were taken place for 0-5 years old children. In EU countries 40% of agricultural production is planned to be turned to organic production. Sweden has made laws for allocating 10% of her current agricultural land to organic production. In addition, Austria aims to raise its proportion of organic agricultural production of total agricultural production to 25% in the coming five years. While these developments are occurring, Turkey having suitable land and rich flora, should rapidly develop an organic production plan. This would enable Turkey to direct her production to the quantities and varieties needed, if she wants to be a

substantial shareholder in the organic product market which is enlarging year to year.

ACKNOWLEDGEMENTS

The authors thank Gregory T. Sullivan of Ondokuz Mayıs University in Samsun, Turkey for his proofreading of this manuscript.

REFERENCES

1. IFOAM, 2006. www.ifoam.org
2. Yussefi, M. and H. Willer, (Eds.), 2005. *The World of Organic Agriculture: Statistics and Emerging Trends 2005*, Germany, IFOAM Publications.
3. Demiryürek, K., 2004. Organic Agriculture and Organic Hazelnut Potential of Black Sea Region, Organic Hazelnut and Tea Potential in the Black Sea Region Symposium, Samsun, pp: 1-2. (in Turkish).
4. Rehber, E. and S. Turan., 2002. Prospects and Challenges for Developing countries in Trade and Production of Organic Food and Fibers: The Case of Turkey, *British Food Journal*, Volume: 104 Issue: 3/4/5, pp: 371-390.
5. Dabbert, S., A.M. Haring and R. Zanoli, 2004. *Organic Farming*, Zed Books Ltd., London.
6. MARA, 2006. www.tarim.gov.tr
7. Demirci, R., A. Erkuş H. Tanrıvermiş E. Gündoğmuş N. Parlitz and H. Özudođru, 2002. Economic Course and Future of Agricultural Crop Production in Turkey: Debate of Forefront Research, Black Sea Region Agricultural Production and Marketing Symposium, October, 15-16, 1999, Black Sea Agricultural Research Institute Publications, Samsun, pp: 197- 210 (in Turkish).
8. REGA, 2004. www.rega.com.tr
9. Bülbül, M. and H. Tanrıvermiş 1999. Economic Structure of Organic and Conventional Hazelnut Production and Export Potential in Turkey, Black Sea Region Agricultural Production and Marketing Symposium, October 15-16, 1999, Black Sea Agricultural Research Institute Publications, Samsun, pp: 144-156 (in Turkish).
10. Güzel, T., 2001. Facilities in the Development of Organic Agricultural Products and Export in Turkey, in *World. Istanbul Chamber of Commerce*, Publication No: 2001-14 İstanbul (in Turkish).
11. IGEME, 2006. www.igeme.org.tr
12. Kayahan, S., 2001. Development of Domestic Market in Organic Agriculture, Second Organic Agriculture Symposium of Turkey, November 14-16, 2001, Antalya, pp: 24-29 (in Turkish).

Risk Assessment for Microbial Pollution in Drinking Water in Small Community and Relation to Diarrhea Disease

A.H. Mahvi and H. Karyab

School of Public Health, Center for Environmental Research,
Tehran University of Medical Sciences, Tehran, IR Iran

Abstract: Object of this study is evaluating of health risk in usage of polluted drinking water in small community, located around of Qazvin, Iran and its relative with prevalence of diarrhea diseases. World health organization has reported that annually 4 billion cases of diarrhea take place world wide, whereas 88 percent of those outbreaks are ascribed to contaminated drinking water [5]. In this study 183 small communities with 10 to 4500 people and total populations of 71171 people were investigated. Results of microbial examinations of drinking water samples, as total coliform, have shown that 73.1 percent of populations have been used contaminated water from march 2005 to February 2006, in 12 months. Investigations in this limited domain were showed that rates of diarrhea outbreak in communities with usage of safe water was 5.3 percent and 8.54 percent in populations with contaminated water. Results were shown that outbreak rates of diarrhea were 69.2 cases in 1000 people in each year and 0.189 in each day. Whereas this rate was 8.94 times fewer than WHO estimated. Also, it was distinguished that rates of diarrhea incidence will be increased with evaluating of environmental temperature. Studying of risk factors was shown that no disinfection had highest role in causing of diarrhea incidence. So in 68 percent of communities chlorination was not performed. It is anticipated that 58605 cases of diarrhea will take place in this domain in next year, if source sanitation and water disinfection do not perform.

Key words: Drinking water % microbial contamination % diarrhea % risk assessment

INTRODUCTION

Epidemiological investigations can provide strong evidence linking exposure to the incidence of diarrhea disease in a population and estimate the magnitude of risk related a particular level of exposure. Also they can specify relation between chance factors and can control risk factors causing gastrointestinal disease [1].

So in this study epidemiology is used as a tool for the assessment of risk. Object of this study is evaluating relation between contaminated water and occurrence of diarrhea in a domain, with usage of epidemiology as a tool for the assessment of risk. Employing risk assessment to control undesirable effects of pollutants on human and environment began before 2 decades ago and it has applied in very cases. Gorter *et al.* [2] has studied effects of water supply and sanitation with outbreak of diarrhea in Nicaragua and they have specified that this rate in children with 500 m distance from source of water in home.

Payment *et al.* [3] have used a randomized controlled trail to investigate whether excess gastroenteritis was being caused by potable water supplies. Result of this study estimated the annual incidence of GI illness among tap- water drinker to be 0.76 versus 0.50 among filtered water drinkers. In addition, the result of this study estimated that 35% of the total reported gastroenteritis among tap- water drinker was water- related [4].

Diarrhea occurs in the world-wide and it causes 4% of all deaths and 5% of health loss to disability. It was estimated each year there are 4 billion cases of diarrhea word wide [5]. Agents of water-related diarrhea are very different. They potentially present in contaminated water and are included bacteria, protozoa and viruses [6, 7]. This study was designed to determine relationship between temperature of environment, rate of contaminated water in each season of year and verifying incidence of diarrhea in each situation. To attain this object, epidemiological methods, especially risk assessment, was employed.

MATERIALS AND METHODS

Number of 183 small community was investigated with populations of 10 to 4500 peoples and total populations of 71171 peoples. Then 507 samples of water that microbiologically examined were investigated due to Total Coliform (TC) and Termo Tolerant Coliform (TTC). To reveal total cases of incidence of diarrhea among one year, data from health and curing centers is collected. To elevate accuracy numbers of peoples polluted to diarrhea, domain of study divided to 10 sub-domains and questionnaires was completed. In this questionnaire information about number of peoples polluted, situation of water supply, source of potable water, disinfection, no disinfection and unsatisfactory control of disinfection was investigated. After that, cases of diarrhea were compared in communities with safe water and contaminated water and incidence of diarrhea. Total samples were grab and selection of sampling places were accidentally. Examinations on samples were achieved on Standard Methods for water and wastewater examinations [8].

RESULTS

To recognize water-related diarrhea outbreak in a domain items such as 1) complaint about water quality, 2) non- potable water found by routine sampling, 3) an increase of GI disease in the community and 4) an increase of positive laboratory result indicating possible waterborne agents, can help [9]. In this study second item guided us. So from 517 samples in 183 small communities, populations in 135 communities had habited in locations with contaminated water, with total populations of 36045 peoples.

Table 1 has shown risk factors causing incidence of diarrhea. No disinfection has highest rate of risk. As in 98% of communities with contaminated water, chlorination is not achieved. Table 2 shows results of qualify analysis of water. Maximum of water contamination take placed in interval of Jun to Aug. In that, indicator of microbiological examinations is total coliform, reproducing of this microorganisms increased in high temperature and so increasing contaminated samples in the warm months will be anticipated [10]. Also rates of diarrhea incidence is shown in this table. Data from this table shows that 60% of diseases take placed in interval Jul of to Oct. Especially, 100% cholera incidences there were in Aug and Sep. In Fig. 1 is shown effects of environmental temperature in contaminating of water. Peak of

Table 1: Effective risk factors in incidence of diarrhea in this study

Risk factors	Parameter		
	Communities with safe water %	Communities with contaminated water %	In total communities %
No disinfection	16.7	98.0	68.0
Unsatisfactory control of disinfection	3.7	0.0	3.0
Breakeage in water supply	10.1	63.2	35.0
No sanitation water source	3.6	46.0	27.0
Wastewater pipes close to water source	16.0	69.3	40.0

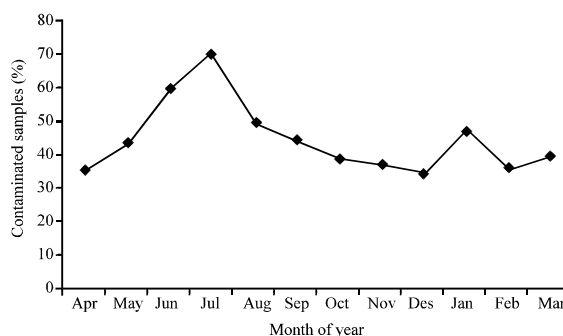


Fig. 1: Effect of season and environment temperature on water quality

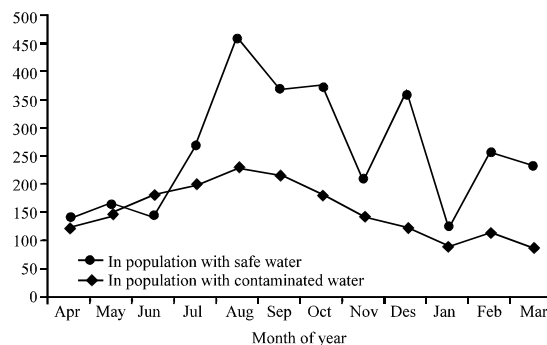


Fig. 2: Numbers of diarrhea diseases in communities with safe and contaminated water

contamination is in Jun to Aug, that have warmest day in this year. In Fig. 2 rates of incidence of diarrhea is compared in communities with safe water and contaminated water.

DISCUSSION

Risk assessment and intervention trials have been used to estimate drinking water health risks. Risk assessment is

Table 2: Results from water examination and Cases of diarrhea incidence

	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Des	Jan	Feb	Mar
Total water sample	43.0	39.0	39.0	40.0	94.0	53.0	49.0	37.0	48.0	23.0	19.0	23.00
Contaminated samples %	34.4	43.5	58.9	70.0	50.0	43.4	38.7	37.0	66.7	47.8	36.8	39.10
Estimated cases of diarrhea	260.0	308.0	325.0	466.0	686.0	581.0	549.0	358.0	482.0	215.0	364.0	320.00
Estimated cases of diarrhea in locations with safe water	120.0	145.0	180.0	198.0	230.0	223.0	180.0	148.0	122.0	89.0	110.0	89.00
Estimated cases of diarrhea in locations with contaminated water	140.0	163.0	145.0	268.0	456.0	368	369.0	210.0	360.0	126.0	254.0	2.31
Fraction from total cases of diarrhea	5.3	6.3	6.6	9.5	10.4	11.8	11.1	7.3	9.8	4.3	7.4	6.50

Table 3: Analyses of occurrence of diarrhea in one year in the selected domain

	Initially reported	Sick identified by questionnaire	Estimated No. of sick	No. at risk (person)
Giardiasis	34*	**	1885	58605
Typhoid	2	**	---	
Dysentery	29	19	190	
Cholera	57	**	---	
Unknown agent	1230	486	4860	

*This number has obtained from examination of 1283 peoples

**No identified from usage the questionnaire

important under conditions of low risk when estimates are difficult to attain from trails [11]. Diarrhea diseases are one of the important agents of mortality, especially in the developing countries P [12]. World health organization has estimated that diarrhea annually 2.2 million people will kill worldwide [5]. Also WHO have reported contaminated water is an important cause of diarrhea. In the world-wide around 1.1 billion people lack access to improved water sources and 2.2 billion have not basic sanitation [13]. Also Lang *et al.* [4] have estimated that 35% of the total reported gastroenteritis among tap- water drinker was water-related.

In this study, estimating are shown that 69.2 cases of diarrhea in each 1000 people have been take placed in each year. This rate is 8.94 times fewer than rate that WHO has estimated. Also result has shown that with increasing temperature in the environment, rate of contaminated water will increased. According to estimations that World Health Organization has done, diarrhea causes 4% of mortalities [5] but in this survey cases of death do not find.

Effect of temperature on rate of contaminated water samples is shown in Fig. 1 maximum percentage of contaminated samples had take placed in warm months. Investigating on Fig. 2 will give two results. The first, rates of incidence of diarrhea in communities with polluted water is higher than communities with safe

water. And the second, with warming of weather, rates of incidence of diarrhea have been increased in each two communities.

REFERENCES

1. Blumenthal, U.J., S.A. Esrey and A. Peasey, 2001. Epidemiology a tool for the assessment of risk. WHO, IWA Publishing
2. Lang, S., L. Fewtrell and J. Bartram, 2001. Risk communication. WHO, IWA Publishing.
3. Knacker, T., 2002. POSEIDON-Environmental risk assessment. European Union Research.
4. Lang, S., L. Fewtrell and J. Bartram, 2001. Risk communication. WHO, IWA Publishing.
5. World Health Organization, 2006. Water sanitation and health, Geneva.
6. Crook, J., 1998. Water reclamation and reuse criteria, Technomic Publishing Co., Ltd. Lancaster, PA.
7. Madigan, M.T., J.M. Martinko and J. Parker, 2000. Brock biology of microorganisms, 9th Ed., Prentice-Hall, Upper saddle river, NJ.
8. Standard Method, 1998. Standard Method for the Examination of Water and Waste Water. 20th Ed., APHS, Washington, DC.
9. Anderson, Y. and P. Bohan, 2001. Disease surveillance and waterborne outbreake. WHO, IWA Publishing
10. Bitton, G., 1999. Wastewater microbiology. Wiley-liss
11. Eisenberg, N.S., A.H. Hubbard, T.J. Wade, M.D. Sylvester, M.W. LeChevallier, D.A. Levy and J.M. Colford, 1991. Inferences Drawn from a risk assessment Compared directly to a randomized trial of a home drinking water intervention. IWA Publishing.
12. Lanata, F.C. and W. Mendoza, 2002. Improving diarrhea estimates. Institute of investigation nutritional, PERU
13. World Health Organization, 2000. Global water supply and sanitation assessment, Geneva.

Feasibility of Solar Energy in Disinfection of Drinking Water in Iran

Amir Hossein Mahvi

School of Public Health, Center for Environmental Researches,
Tehran University of Medical Sciences, Tehran, Iran

Abstract: The solar disinfection of water (SODIS) is a simple technique used to destroy pathogenic microorganisms and so it can improve microbiological quality of drinking water. Many countries are in good positions with respect to receiving solar radiation and Iran ranks first in this regard. Thus, it is important for health authorities to prefer this simple method for use in rural areas of the Country and at abnormal conditions instead of other complicated techniques. The main objective of this study was to determine the efficiency of locally available bottles (not transparent to UVC and semi-transparent to UVA) for use in solar disinfection of water in non-urban areas of Iran. For this purpose normal plastic bottles were used and the solar disinfection efficiency was evaluated in terms of fecal coliform reduction of contaminated surface water samples. Two types of locally available normal plastic bottles with UV transmittance values of 0.1 and 0.8 percent were selected and used according to WHO guidelines about SODIS in the disinfection process of water samples from a surface water resource. Examinations of microbiological quality of all water samples have been performed by determination of fecal coliform group (5 tube fermentation technique) according to the procedure outlined in Standard Methods. Water sampling had been accomplished in the fall of 2006. Results indicate that SODIS is also possible even if available plastic bottles with less transparency are used instead of standard bottles. According to the results obtained by use of these bottles, about 99.9% disinfection of water (up to 3 log reduction in fecal coliforms) is possible at the temperature of 39.6 degree centigrade. Also, it should be noted that by substituting the bottle with less UVT with the more transparent one, it would be possible to decrease the required contact time for 3 log₁₀ reduction of microbial indicator from 8 to about 6 hours. Results of this study clearly indicate that utilizing of both locally available bottles used in this study may have enough justification for SODIS process in non-urban areas and communities of Iran which mostly have warm climates.

Key words: Drinking water % disinfection % solar radiation % plastic bottles % non-urban areas

INTRODUCTION

No resource is as universally necessary to sustain life as is safe drinking water. But water used for drinking and food preparation has also been responsible for transmission of numerous infections agents. Diarrhea diseases which mainly result from drinking water that has been contaminated through unsafe disposal of sewage are among the top 3 causes of death world wide and the leading cause of death among children under 5 in most developing countries. Thereupon, all governments should promote equitable access to safe water supplies and strengthen their programs to improve water quality.

Solar disinfection, or SODIS as it is known, is one of the simplest methods for providing acceptable quality drinking water. The SODIS technique involves storing

contaminated drinking water in transparent containers (plastic bags, plastic bottles or glass bottles) that are placed in direct sunlight for periods of up to 8 h before consumption[1,2]. Pathogenic microorganisms are vulnerable to two effects of the sunlight: radiation in the spectrum of UV-A light (wavelength of 320-400 nm) and heat (increased water temperature). A synergy of these two effects occurs, as their combined effect is much greater than the sum of the single effects. This means that the mortality of the microorganisms increases when they are exposed to both temperature and UV-A light at the same time [3].

SODIS is ideal to disinfect small quantities of water of low turbidity. If cloudiness of pathogens is greater than 50%, the plastic bottles need to be exposed for 2 consecutive days in order to produce

water safe for consumption. However, if water temperatures exceed 50°C, one hour of exposure is sufficient to obtain safe drinking water. The treatment efficiency can be improved if the plastic bottles are exposed on sunlight reflecting surfaces such as aluminium or corrugated iron sheets [7, 8].

The other important factors include latitude of location, turbidity of water and dissolved oxygen of water and bottle type which all affect the disinfection efficiency of SODIS [3, 6].

The effectiveness of SODIS in reduction of various microorganisms was studied in the recent years. The results of the investigations show that this technique is highly effective against a broad range of bacterial fungal and free-living protozoan pathogens such as *Vibrio cholerae* [2, 7], *Salmonella typhimurium* [8], *Shigella dysenteriae* type I [7], *Pseudomonas aeruginosa*, *Candida albicans*, *Fusarium solani* and the trophozoite stage of *Acanthamoeba polyphaga* [9] and *Cryptosporidium parvum* [6, 7, 10, 11]. Previous studies have reported a reduction in incidence of diarrhea among those children who drank water exposed to direct sunlight compared with another group that drank water not exposed to sunlight [2].

There are a few criteria that must be applied in selecting the appropriate type of containers to be used for the proper disinfection of contaminated drinking water by sunlight. The important rule to be followed is to base the selection not only on availability and size, but also on the need to use containers that would permit the penetration of sun rays. However, for many regions and under abnormal conditions, accessible containers are often used without attention to this subject.

The objective of this research was to study the feasibility of SODIS application in the rural areas of Iran. In our study, two types of locally available normal plastic bottles were used as the possible containers and the efficiency of SODIS was investigated in terms of reduction in fecal coliforms indicator of contaminated surface water samples.

METHODS

Water sampling had been performed from a surface water canal in Tehran. The experiments were done in fall season, the average air temperature was about 17°C and the sky was relatively clear. Aeration of the water was achieved by shaking the three fourth filled bottles for about 20 seconds before the bottle was filled completely and exposed to the sun. Fecal coliforms indicator was examined for determination of SODIS

efficiency. Therefore, the number of fecal coliforms in the water samples was measured before and after solar exposure. The number of fecal coliforms was determined as most probable number per 100mL (MPN/100 mL) using 15-tube fermentation technique according to the procedure outlined in "Standard Methods for the Examination of Water and Wastewater"[12]. The initial turbidity and fecal coliforms of water samples were about 1 NTU (Nephelometric Turbidity Units) and 2000-3000 MPN/100 mL, respectively.

The volume of normal plastic bottles used in the research was 1.5 L. The effect of solar radiation time on SODIS efficiency was investigated at 3, 6 and 8 h exposure times. Also, the effect of transparency of bottles for UV radiation was studied using two types of normal plastic bottles with 0.1 and 0.8 percent UV transmittance at the wavelength of 254 nm. All of the experiments were performed in triplicate and the average values were presented.

RESULTS

The effect of solar radiation time on SODIS disinfection efficiency is presented in Fig. 1. For those experiments the normal plastic bottles with 0.1 percent UV transmittance (at the wavelength of 254 nm) had been used. The average water temperature was 19°C at the beginning of the experiments; also the average water temperature was obtained to be 39, 40 and 38°C after 3, 6 and 8 h radiation time, respectively. As Fig. 1 illustrates, SODIS efficiency in fecal coliforms reduction was determined to be 93, 99.8 and 99.9 percent at 3, 6 and 8 h radiation time, respectively. Therefore, this means that for 3 log₁₀ reduction of fecal coliforms a relatively long radiation time (8 hours) was required.

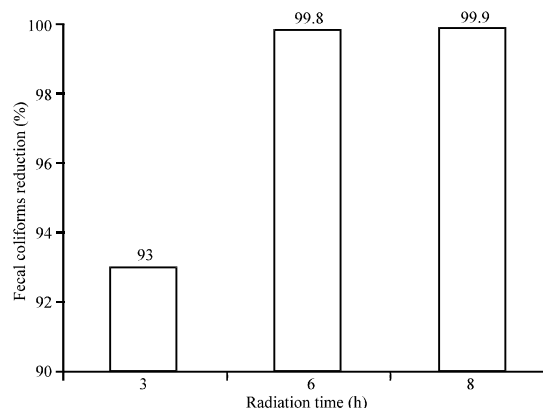


Fig. 1: Effect of solar radiation time on SODIS disinfection efficiency

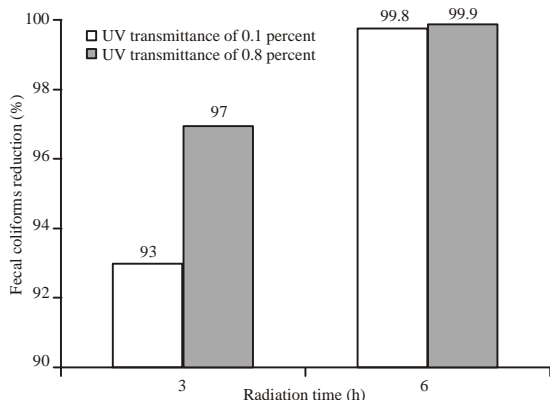


Fig. 2: Effect of UV transmittance of bottles on SODIS disinfection efficiency

Figure 2 illustrates the effect of UV transmittance of bottles on SODIS efficiency. The average water temperature was 20°C at the beginning of the experiments; also the average water temperature was obtained to be 41 and 40 degrees centigrade after 3 and 6 h radiation time, respectively. According to Fig. 2, the required radiation time for 3-log reduction of fecal coliforms had been decreased to 6 h by using more transparent bottle.

DISCUSSION

The disinfection efficiency of SODIS was considerable, such that 3-log reduction of fecal coliforms was achieved at 6 to 8 h radiation time by using normal plastic bottles with 0.1 and 0.8 percent UV transmittance. It is also anticipated that by application of more transparent bottles, the required exposure time would be less than 8 hours. However, for household applications of the technique, the environmental factors affecting SODIS should be studied.

The efficiency of the SODIS process is dependent on the amount of sunlight available. Solar radiation however is unevenly distributed and varies in intensity from one geographical location to another depending on latitude, season and the time of the day [13, 14]. Iran is located between latitude of 25°N and 40°N. This means that the country is in a good position. Besides, sunshine duration is also suitable for SODIS, especially in the central and southern parts of Iran, so over 90% of the sunlight directly touches the earth due to the limited cloud cover and rainfall (less than 250mm rain and usually more than 3000 hours of sunshine annually).

It is obvious that the negative effect of using containers with less UVT would be in producing water with less quality. However, at contact times equal or more

than 6 hours this effect would not be a problem and as Fig. 2 shows water disinfection is accomplished quite well by both containers.

Turbidity of water decreases the penetration of solar radiation into water and protects microorganisms from being irradiated. Therefore, the disinfection efficiency of SODIS is reduced in turbid water [4, 15]. In the rural areas of Iran, drinking water is usually provided from groundwater resources such as qanats, springs and wells. Generally, turbidity of the groundwater resources is low. Therefore, the water quality most parts of the country is suitable for SODIS application.

The study on SODIS efficiency and environmental factors affecting SODIS including climate and turbidity of water indicated that SODIS technique is an appropriate method for household water disinfection in rural areas of Iran. Consequently, it is recommended that training of SODIS application is set in health education program in the rural areas without access to safe water.

REFERENCES

1. Wegelin, M., S. Canonica, K. Mechsner, T. Fleischmann, F. Pesaro and A. Metzler, 1994. Solar water disinfection: scope of the process and analysis of radiation experiments. *J. Water SRT. Aqua.*, 43: 154-169.
2. Conroy, R.M., M.E. Meegan, T. Joyce, K. McGuigan and J. Barnes, 2001. Solar disinfection of drinking water protects against *cholera* in children under 6 years of age. *Arch. Dis. Child*, 85: 293-295.
3. Reed, R.H., S.K. Mani V. Meyer, 2000. Solar photo-oxidative disinfection of drinking water: preliminary field observations. *Lett. Appl. Microbiol.*, 30: 432-436.
4. McGuigan, K.G., T.M. Joyce, R.M. Conroy, J.B. Gillespie and M. Elmore, 1998. Solar disinfection of drinking water contained in transparent plastic bottles. *J. Appl. Microbiol.*, 84: 1138-1148.
5. Fujioka, R.S. and B.S. Yoneyama, 2002. Sunlight inactivation of human enteric viruses and fecal bacteria. *Water Sci. Technol.*, 46: 291-295.
6. Clancy, J.L., M.M. Marshal, T.M. Hargy and D.G. Korich, 2004. Susceptibility of five strains of *Cryptosporidium parvium* oocysts to UV light. *J. AWWA*, 96: 84-93.
7. Kehoe, S.C., M.R. Barer, L.O. Devlin and K.G. McGuigan, 2004. Batch process solar disinfection is an efficient means of disinfecting drinking water contaminated with *Shigella dysenteriae* type I. *Lett. Appl. Microbiol.*, 38: 410-414.

8. Smith, R.J., S.C. Kehoe, K.G. McGuigan and M.R. Barer, 2000. Effects of simulated solar disinfection of water on infectivity of *Salmonella typhimurium*. *Lett. Appl. Microbiol.*, 31: 284-288.
9. Lonnen, J., S. Kilvington, S.C. Kehoe, F. Al-Touati and K.G. McGuigan, 2005. Solar and photocatalytic disinfection of protozoan, fungal and bacterial microbes in drinking water. *Water Res.*, 39: 877-883.
10. Mendez-Hermida, F., J.A. Castro-Hermida and M.E. Ares, 1998. Effect of batch process solar disinfection on *Cryptosporidium parvum* oocysts in drinking water. *Appl. Environ. Microbiol.*, 71: 1653-1654.
11. Mofidi, A.A., H. Baribeau, P.A. Rochelle, R.D. Leon, B.M. Coffey and J.F. Green, 2001. Disinfection of *Cryptosporidium parvum* with polychromatic UV light. *J.AWWA*, 93: 95-109.
12. APHA, AWWA, WPCF. 1998. Standard methods for the examination of water and wastewater. 20th ed, Maryland, United Book Press,
13. Masschelein, W.J., 2002. UV light in water and wastewater sanitation. Boca Raton, CRC Press,
14. Kehoe, S.C., T.M. Joyce, P. Ibrahim, J.B. Gillespie, R.A. Shahar and K.G. McGuigan, 2001. Effect of agitation, aluminum foil reflectors and volume on inactivation of efficiency of Batch-process solar disinfectors. *Water Res.*, 35: 1061-1065.
15. Rose, A., S. Roy, V. Abraham, G. Holmgren and K. George, 2006. Solar disinfection of water for diarrhoeal prevention in India. *Arch. Dis. Child*, pp: 139-141.

Natural Forest and Forest Plantation Affect Diversity of Arbuscular Mycorrhizal Fungi in the Rhizosphere of Dipterocarpaceae

¹Zhao Yong Shi, ¹Fa Yuan Wang and ²Yan Li Wei

¹Department of Resources and Environmental Science, College of Agriculture, Henan University of Science and Technology, Luoyang, Henan Province, 471003, P.R. China

²Institute of Biology, Shandong Academy of Sciences, Key Laboratory of Applied Microbiology of Shandong Province, Jinan, Shandong Province, 250014, P.R. China

Abstract: The influences of natural forest and forest plantation of Dipterocarpaceae on arbuscular mycorrhizal (AM) fungal diversity were studied based on the investigation of *Hopea hainanensis* and *Vatica astrotricha* of Dipterocarpaceae grown in natural forest and forest plantation in Jianfengling Mountain of Hainan Island in South of China. The results showed that the percentage of root length colonized by AM fungal structures, spore density and species diversity of AM fungi were all higher in natural forest than in forest plantation. AM fungal species richness of *Hopea hainanensis* was significantly higher in natural forest than in forest plantation. However, species richness in the rhizosphere of *Vatica astrotricha* wasn't significantly different between natural forest and forest plantation. Twenty-one AM fungal species belonging to five genera were isolated and identified in rhizosphere soils of the two objective plants. Among them, 14 species are belonging to genus of *Glomus*, 4 for *Acaulospora* and 1 for each of *Archaeospora*, *Gigaspora* and *Scutellospora* respectively. *Glomus* species are found to be the dominant AM fungi in either natural forest or forest plantation.

Key words: Arbuscular mycorrhizal fungi % Dipterocarpaceae % diversity % natural forest % forest plantation

INTRODUCTION

As the most Widespread Symbiosis on Earth [1], Arbuscular mycorrhizal (AM) fungi evolved concurrently with the first colonization of land by plants some 450 to 500 million years ago and persist in most extant plant taxa [2]. The associations formed between plant roots and AM fungi are of great interest because of their potential influence on ecosystem processes, their role in determining plant diversity in natural communities and the capacity of AM fungi to induce a wide variety of growth responses in coexisting plant species [3-7]. Recent studies have indicated that AM fungi are common and ecologically important in tropical ecosystems and that cooccurring plant species vary considerably in their germination, growth and flowering responses to mycorrhizal colonization along a continuum from highly responsive obligately mycotrophic species to facultatively mycotrophic and nonresponsive species [8, 9]. Tropical rain forests display high plant species diversity and complex community structure [10].

The Dipterocarpaceae is one of the most important tree families in tropical rainforests both ecologically and economically. It is the symbol of tropical rainforest. Some trees are a major source of very valuable tropical hardwood timber such as *Hopea chinensis* and members of the family are also used for resin and gums. In recent years, there has been increasing interest in the arbuscular mycorrhizae of tropical rain forest plants including natural forests [11-13], secondary forest [14] and deforested forest [15]. Especially, the Arbuscular mycorrhizal fungi associated with Dipterocarpaceae also intrigued several researchers [13, 16]. As a kind of soil microorganisms, AM fungi do not avoid the influence of vegetation types [15, 17]. Zhang *et al.* have shown that the diversity of AM fungi were different in deforested and natural forest lands [15]. However, the effect of natural forest and forest plantation on diversity of AM fungi lacked systematic study, though there are fragmentary reports [13]. The purpose of present study is to investigate the effect of natural forest and forest plantation on AM fungal diversity by comparing the differences AM fungal

diversity in the rhizosphere of natural forest and forest plantation of Dipterocarpaceae based on a broad field survey in Jianfengling Mountain of Hainan Island in South of China.

MATERIALS AND METHODS

Site location: Jianfengling, located in the juncture of Ledong and Dongfang counties, is on the southwestern part of Hainan Island at 18°23'-18°52' N and 108°46'-109°02' E. The total area of Jianfengling forest region is 47227 km² and this makes the region one of the 5 largest forest areas on the island. The highest elevation of Mt. Jianfengling Peak is 1412.5 m. Jianfengling Primeval Tropical Forest is one of China's biggest and best-preserved primeval tropical forest areas. The samples of *Hopea hainanensis* and *Vatica astrotricha* in natural forest were collected from the Mt. Jianfengling Peak with the altitude of around 850 m and 500 m, respectively. The samples in forest plantation were collected from Jingfengling farms.

Collection of soil and root samples: Surface soil (approximately 1–2 mm) was removed and soil cores of 0 to 50 cm were collected including fine roots and rhizosphere soils of the host plants. Root samples were collected from a total of 2 objective species of *Hopea hainanensis* and *Vatica astrotricha* of Dipterocarpaceae. Roots were traced back to the stem of the host plants to ensure that the roots were indeed connected to the plants selected for sampling. Three rooting-zone soil samples (each approximately 1000 g) with fine roots were collected in three different directions from each plant and the three samples were mixed thoroughly. A subsample of approximately 500 g was then taken for assessment of AM fungal colonization and extraction of AM fungal spores. Six individuals of each plant species were randomly selected for sampling of soil and roots. Samples of plant roots were taken to the laboratory for determination of root colonization. The soil samples were then air-dried in the shade at laboratory temperature (10-26°C) for spore extracting, counting and identification.

Assessment of AM colonization: Fresh roots were processed by washing them to get rid of adhering soil particles and clearing in 10% (w/v) KOH: distilled water at 90°C in a water bath for 30–60 min, the exact time depending on the degree of lignification of the roots and their pigmentation. The cooled root samples were washed and cut into 0.5 to 1.0-cm-long segments and stained

with 0.5% (w/v) acid fuchsin [18]. The percentage of root length colonized by AM fungal structures was determined using the magnified line-intersect method of McGonigle *et al.* [19].

Recovery and counting of AM fungal spores: Spores or sporocarps were extracted from 20 g air-dried subsamples of each soil sample in triplicate by wet sieving (53 µm) followed by flotation–centrifugation in 50% sucrose [20]. The spores were collected on a grid patterned (4×4 mm) filter paper, washed three times with distilled water to spread them evenly over the entire grid and counted using a dissecting microscope at 30× magnification. A sporocarp was counted as one unit. For observation and identification of spore characters, spores were mounted on glass slides in polyvinyl alcohol–lactoglycerol (PVLG) and PVLG + Melzer's reagent and then identified to species level using current taxonomic criteria [21] and information published by INVAM (<http://www.invam.caf.wvu.edu>).

Numbers and distribution of AM fungal spores: Spore density and species richness of AM fungi were expressed as follows:

$$\text{Spore density} = \text{No. of AM fungal spores in 20 g dry soil} \quad (1)$$

$$\text{Species richness} = \text{No. of AM fungal taxa found in 20 g dry soil} \quad (2)$$

Species diversity of AM fungi was assessed by the Shannon–Weiner index as follows:

$$\text{Shannon-Weiner index} = -\sum (P_i \ln[P_i]) \quad (3)$$

where $P_i = n_i/N$ and n_i = number of individuals in species i ; N is the total number of individuals in all species.

Statistical analysis: The data were subjected to one-way ANOVA using SPSS software version 11.0. The differences in percent root length colonized, spore density, species richness and species diversity were separated by least significant difference (LSD) test for significantly different means in all taxa.

RESULTS

Effect of natural forest and forest plantation on AM fungal colonization: The AM fungal colonization of *Hopea hainanensis* and *Vatica astrotricha* of

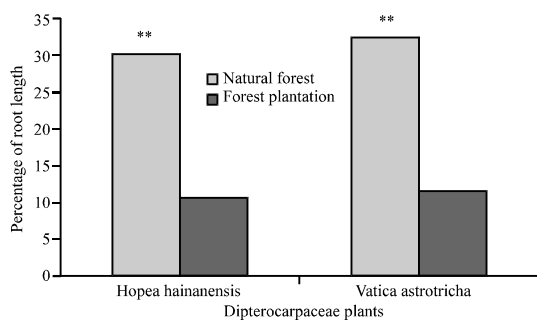


Fig. 1: Effect of natural forest and forest plantation on AM fungal colonization

Note: ** means significant difference at the level of $p=0.01$.

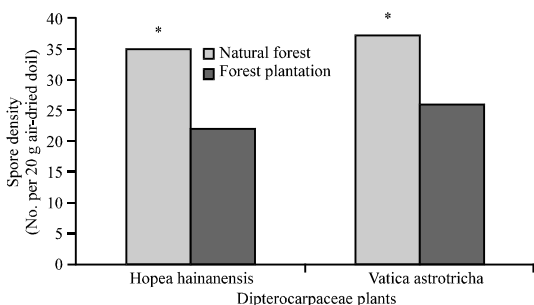


Fig. 2: Effect of natural forest and forest plantation on AM fungal spore density

Note: * means significant difference at the level of $p=0.05$.

Dipterocarpaceae in natural forest and forest plantation are presented in Fig. 1. The percentage of root length colonized of *Hopea hainanensis* and *Vatica astrotricha* are significantly higher in natural forest than in forest plantation.

Effect of natural forest and forest plantation on AM fungal spore density: The spores of AM fungi of 20 ml air-dried soil in rhizospheres of *Hopea hainanensis* and *Vatica astrotricha* of Dipterocarpaceae are isolated from natural forest and forest plantation, respectively. The spore densities of AM fungi are significantly higher in natural forest than in forest plantation in the rhizosphere either *Hopea hainanensis* or *Vatica astrotricha* (Fig. 2).

Effect of natural forest and forest plantation on AM fungal species richness: Figure 3 indicated the species richness of AM fungi in rhizospheres of *Hopea hainanensis* and *Vatica astrotricha* of Dipterocarpaceae in natural forest and forest plantation. AM fungal species richness in the rhizosphere of *Hopea hainanensis* is

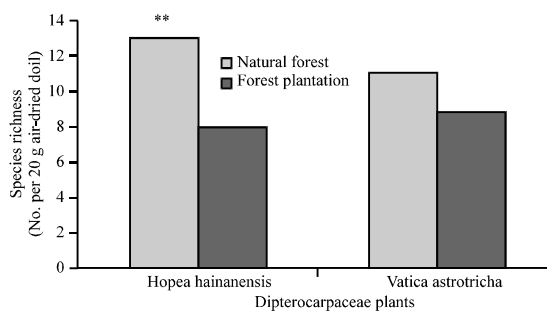


Fig. 3: Effect of natural forest and forest plantation on AM fungal species richness

Note: ** means significant difference at the level of $p=0.01$.

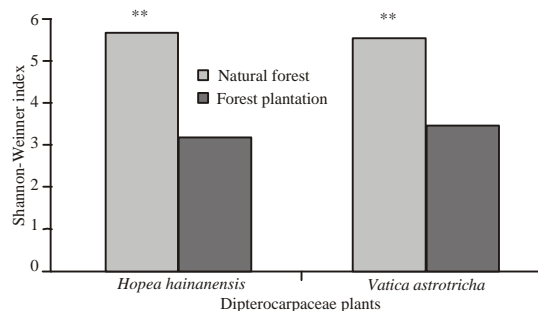


Fig. 4: Effect of natural forest and forest plantation on AM fungal species diversity

Note: ** means significant difference at the level of $p=0.01$.

higher in natural forest than in forest plantation significantly. However, no significant difference was observed in the rhizosphere of *Vatica astrotricha* between natural forest and forest plantation.

Effect of natural forest and forest plantation on AM fungal species diversity: The influences of natural forest and forest plantation on AM fungal species diversity were analyzed based on *Hopea hainanensis* or *Vatica astrotricha* (Fig. 4). The results showed that the diversity was more abundant in natural forest than in forest plantation in the rhizosphere either *Hopea hainanensis* or *Vatica astrotricha*.

Effect of natural forest and forest plantation on AM fungal community composition: Twenty-one AM fungal species belonging to five genera, including one species in *Archaeospora*, four in *Acaulospora*, one in *Gigaspora*, fourteen in *Glomus* and one in *Scutellospora*, were isolated and identified in the rhizosphere of *Hopea hainanensis* and *Vatica astrotricha* in natural forest and forest plantation (Table 1). Fifteen species representing

Table 1: Effect of natural forest and forest plantation on AM fungal community composition

AM fungi	Natural forest	Forest plantation
<i>Acaulospora appendicola</i> Rothwell and Trappe	# +	# +
<i>A. denticulate</i> Sieverd. and Toro	#	#
<i>A. foveata</i> Trappe and Janos	+	#
<i>A. rehmi</i> Sieverd. and Toro	#	+
<i>Archaeospora leptoticha</i> (Schenck and Sm.) Morton and Redecker	#	#
<i>Gigaspora margarita</i> Becker and Hall	#	
<i>Glomus aggregatum</i> Schenck and Sm.	# +	# +
<i>G. caledonium</i> (Nicolson and Gerd.) Trappe and Gerd.	# +	# +
<i>G. chimonobambusa</i> Wu and Liu		#
<i>G. claroideum</i> Schenck and Sm. emend Walker and Vestberg	# +	+
<i>G. deserticola</i> Trappe, Bloss and Menge	#	#
<i>G. constrictum</i> Trappe	# +	+
<i>G. etunicatum</i> Becker and Gerd.	#	# +
<i>G. geosporum</i> (Nicol. and Gerd.) Walker	+	
<i>G. hoi</i> Berch and Trappe	+	
<i>G. macrocarpum</i> Tul. and Tul.	#	+
<i>G. microaggregatum</i> Koske, Gemma and Olexia	# +	# +
<i>G. microcarpum</i> Tul. and Tul.	+	+
<i>G. mosseae</i> (Nicol. and Gerd.) Gerd. and Trappe	#	# +
<i>G. reticulatum</i> Bhattacharjee and Mukerji	+	
<i>Scutellospora aurigloba</i> Walker and Sanders	# +	+

Note: # present in the rhizosphere of *Hopea hainanensis*. + present in the rhizosphere of *Vatica astrotricha*.

5 genera AM fungi presented in rhizosphere of *Hopea hainanensis* in natural forest comparing to 12 fungal species within 3 genera in forest plantation. As to AM fungi associated with *Vatica astrotricha*, the same number species presented in natural forest and forest plantation. They included 12 species in *Acaulospora*, *Glomus* and *Scutellospora*.

DISCUSSION

Most trees in tropical forest might be associated with arbuscular mycorrhizas [9, 11, 12, 22-25]. As a symbol of tropical rainforest, the AM fungal diversity associated with Dipterocarpaceae plants attracted more attention of many researchers [13, 16, 26, 27]. Furthermore, AM fungal diversity, e.g., spore formation, distribution, species community composition and mycorrhizal development, is affected by vegetation types or life formations [15, 17, 28]. The present study has indicated natural forest and forest plantation affected diversity of AM fungi in the Rhizosphere of Dipterocarpaceae.

The percentage of root length colonized by AM fungal structures, spore densities and species diversities connected with *Hopea hainanensis* and

Vatica astrotricha were higher in natural forest than in forest plantation. This is in agreement with the previous reports [13]. The possible reasons are that although the tree species selected are same, the annual or perennial herbaceous plant species existed more in natural forest than in forest plantation. These annual or perennial herbaceous plants, as AM fungal hosts, are associated with more AM fungal spore production than are evergreen broad-leaved trees [29, 30]. Moreover, the plant diversity affected the diversity of AM fungi [31-33]. As to the species richness of AM fungi, the significant difference was observed in rhizosphere of *Hopea hainanensis* between natural forest and forest plantation. However, species richness associated with *Vatica astrotricha* wasn't significant different between natural forest and forest plantation. The possible explanation is that the samples collecting error caused the results. This result was presented by species community composition in Table 1. As far as Figure 3 of species richness associated with *Vatica astrotricha* is concerned, although there were no significant differences between natural forest and forest plantation as statistical analysis stated, Figure 3 showed the species richness in natural forest is little higher than in forest plantation. However,

Table 1 indicated the same number of species were isolated and identified in the rhizosphere of *Vatica astrotricha* between natural forest and forest plantation. Figure 3 and Table 1 seem incompatible, but in fact they are accordant, because the data in Fig. 3 are the average of six random tree individuals, while the data in Table 1 are the maximal data presented in the rhizosphere of *Vatica astrotricha* six random tree individuals.

Twenty-one AM fungal species representing five genera were isolated from the rhizosphere soils of *Hopea hainanensis* and *Vatica astrotricha* in natural forest and forest plantation. Fourteen *Glomus* species appeared to be dominant in the rhizosphere soils of *Hopea hainanensis* and *Vatica astrotricha* in natural forest and forest plantation in Jianfengling Mountain. In contrast, *Acaulospora*, *Archaeospora*, *Gigaspora* and *Scutellospora* represented only 19.0, 4.8, 4.8 and 4.8% of the species present, respectively. This provides strong support for the conclusions drawn by other workers who have suggested that *Glomus* species tend to be the dominant AM fungi in tropical rainforest ecosystems [11-13]. In addition, the genus *Glomus* is the largest of all the AM fungal genera in the Glomales [34].

Additionally, not only the forest type but seasonality, host-dependence and age of the host plants, etc. of Dipterocarpaceae can influence the AM fungal colonization, spore density, species richness and diversity and species community composition. These aspects still need further research.

ACKNOWLEDGEMENTS

The project was financially supported by Doctoral Foundation of Henan University of Science and Technology (06-34).

REFERENCES

1. Brachmann, A. and M. Parniske, 2006. The most widespread symbiosis on earth. *PloS Biol.*, 4: 1111-1112.
2. Cairney, J.W.G., 2000. Evolution of mycorrhiza systems. *Naturwissenschaften*, 87: 467-475.
3. Sanders, I.R., J.P. Clapp and A. Wiemken, 1996. The genetic diversity of arbuscular mycorrhizal fungi in natural ecosystems: a key to understanding the ecology and functioning of the mycorrhizal symbiosis. *New Phytol.*, 133: 123-134.
4. Van der Heijden, M.G.A., T. Boller, A. Wiemken and I.R. Sanders, 1998. Different arbuscular mycorrhizal fungi species are potential determinants of plant community structure. *Ecology*, 79: 2082-2091.
5. Van der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Weimken and I.R. Sanders, 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396: 69-72.
6. Hartnett, D.C. and G.W.T. Wilson, 1999. Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology*, 80: 1187-1195.
7. Klironomos, J.N., J. McCune, M. Hart and J. Neville, 2000. The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecol Lett*, 3: 137-141.
8. Johnson, N.C., J.H. Graham and F.A. Smith, 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytol.*, 135: 575-586.
9. Muthukumar, T., L.Q. Sha, X.D. Yang, M. Cao, J.W. Tang and Z. Zheng, 2003. Mycorrhiza of plants in different vegetation types in tropical ecosystems of Xishuangbanna, southwest China. *Mycorrhiza*, 13: 289-297.
10. Read, D.J., 1994. Plant-microbe mutualisms and community structure. In: Schulze E.D. and H.A. Mooney (eds.). *Biodiversity and ecosystem function*. Springer, Berlin Heidelberg New York, pp: 181-209.
11. Zhao, Z.W., Y.M. Xia, X.Z. Qin, X.W. Li, L.Z. Cheng, T. Sha and G.H. Wang, 2001. Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rain forest of Xishuangbanna, southwest China. *Mycorrhiza*, 11: 159-162.
12. Shi, Z.Y., Y.L. Chen, G. Feng, R.J. Liu, P. Christie and X.L. Li, 2006. Arbuscular mycorrhizal fungi associated with the Meliaceae on Hainan island, China. *Mycorrhiza*, 16: 81-87.
13. Shi, Z.Y., F.Y. Wang and Y.L. Wei, 2007. Observations of Arbuscular Mycorrhizas on Dipterocarpaceae Grown in Tropical Rainforest in China. *Am-Euras. J. Agric. & Environ. Sci.*, 2: 247-254.
14. Fang, H., P.N. Damodaran and M. Cao, 2006. Arbuscular mycorrhizal status of *Glomus* plants in tropical secondary forest or Xishuangbanna, Southwest China. *Acta Ecol. Sin.*, 26: 4179-4185.

15. Zhang, Y., L.D. Guo and R.J. Liu, 2004. Survey of arbuscular mycorrhizal fungi in deforested and natural forest land in the subtropical region of Dujiangyan, southwest China. *Plant and Soil*, 261: 257-263.
16. Tawaraya, K., Y. Takaya, M. Turjaman, S.J. Tuah, S.H. Limin, Y. Tamai, J.Y. Cha, T. Wagatsuma and M. Osaki, 2003. Arbuscular mycorrhizal colonization of tree species grown in peat swamp forests of Central Kalimantan, Indonesia. *Forest Ecol. & Manag.*, 182: 381-386.
17. Fisher, M.A. and P. Z. Fulé, 2004. Changes in forest vegetation and arbuscular mycorrhizae along a steep elevation gradient in Arizona. *Forest Ecol. & Manag.*, 200: 293-311.
18. Biemann, B. and R.G. Linderman, 1981. Quantifying vesicular arbuscular mycorrhizae: a proposed methods towards standardization. *New Phytol.*, 87: 63-67.
19. McGonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild and J.A. Swan, 1990. A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytol.*, 115: 495-501.
20. Dalpé, Y., 1993. Vesicular–arbuscular mycorrhiza. In: Carter M.R. (ed.). *Soil sampling and methods of analysis*. Lewis Publishers, Boca Raton, FL., pp: 287-301.
21. Morton, J.B. and D. Redecker, 2001. Two new families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera Archaeospora and Paraglomus, based on concordant molecular and morphological characters. *Mycologia*, 93: 181-195.
22. Alexander, I.J., 1989. Systematics and ecology of ectomycorrhizal legumes. *Monographs in Systematics Botany from the Missouri Botanical Garden*, 29: 607-624.
23. Hart, T.B., 1989. Monodominant and species-rich forests of the humid tropics: causes for their co-occurrence. *Am. Naturalist*, 133: 613-633.
24. Bereau, M., 1997. Les symbioses des arbres de la foret tropicale humide de Guyane francaise. *Can. J. Bot.*, 75: 711-716.
25. Henkel, T.W., 1999. New taxa and distribution records of *Tylopilus* from *Dicymbe* forests of Guyana. *Mycologia*, 91: 655-665.
26. Shamsudin, M.N., 1979. Mycorrhiza of tropical forest trees. In: Furtado, J.I. (ed.). *V International Symposium of Tropical Ecology*. Kuala Lumpur, Malaysia, pp: 173.
27. Chalermpongse, A., 1987. Mycorrhizal survey of Dry-Deciduous and Semi-Evergreen Dipterocarp forest ecosystems in Thailand. In: Kostermans, A.C.J.H. (ed.). *Proceedings of the Third Round Table Conference on Dipterocarps*. Mulawarman University, East Kalimantan.
28. Tian, C.Y., Z.Y. Shi, Z.C. Chen and G. Feng, 2006. Arbuscular mycorrhizal associations in the Gurbantunggut Desert. *Chin. Sci. Bul.*, 51: 1140-1146.
29. Hetrick, B.A.D. and J. Bloom, 1986. The influence of host plant on production ability of vesicular-arbuscular mycorrhizal spores. *Mycologia*, 78: 32-36.
30. Trappe, J.M., 1987. Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In: Safir G.R. (ed.). *Ecophysiology of VA Mycorrhizal Plants*. CRC Boca Raton, Fla., USA., pp: 5-26.
31. Eom, A., D.C. Hartnett and G. Wilson, 2000. Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. *Oecologia*, 122: 435-444.
32. Husband, R., E.A. Herre, S.L. Turner, R. Gallery and J.P.W. Young, 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. *Mol. Ecol.*, 11: 2669-2678.
33. Zak, D.R., W.E. Holmes, D.C. White, A.D. Peacock and D. Tilman, 2003. Plant diversity, soil microbial communities and ecosystem function: Are there any links? *Ecology*, 84: 2042-2050.
34. Schwarzott, D., C. Walker and A. Schüßler, 2001. *Glomus*, the largest genus of the arbuscular mycorrhizal fungi (Glomales), is nonmonophyletic. *Mol. Phyl. Evol.*, 21: 190-197.

The Effects of Water Deficit During Growth Stages of Canola (*Brassica napus* L.)

¹Jafar Masoud Sinaki, ²Eslam Madjidi Heravan, ³Amir Hossein Shirani Rad,
⁴ghorban Noormohammadi and ⁵Ghasem Zarei

¹Department Student of Crop Physiology, Islamic Azad University,
Science and Research Branch, Tehran, Iran

²Department of Plant Breeding, Plant Biotechnology Institute, Karaj, Iran

³Department of Agronomy, Oil Seed Crops Institute, Karaj, Iran

⁴Department of Agronomy, Islamic Azad University, Science and Research Branch, Tehran, Iran

⁵Department of Irrigation, IWMI Institute, Tehran, Iran

Abstract: This study was carried out in farm of the department of agronomy Islamic Azad University science and research branch, in 2004-5 and 2005-6 to determine the effect of different forms of irrigation on the canola (*Brassica napus* L.) Yield and yield components, seed oil and protein content. Ebonit, Elite and SLM046 Cultivars were planted pot experiments under eight treatments including short and long periods of water stress during different growth stages. The greatest seed yield reduction was observed when water stress occurred at flowering (30.3%) and then at silique development (20.7%). Seed yield reduction by short-term water stresses during stem elongation, flowering and silique development were mostly associated with the reduction of silique number per plant but by short-term water stress during seed development was due to the reduction of seed weight. A little compensation was observed by seed weight when water stress occurred before flowering. The number of siliques per plant was the most sensitive yield components under long-term water stress. Seed oil content was decreased by water stress but protein content increased.

Key words: Water deficit % *Brassica napus* L % growth stages % yield and yield components % physiological traits

INTRODUCTION

The world is facing serious shortages of fresh water and growing competition for clear water makes less water available for agriculture. The great challenge for the coming decades will be the task of increasing food production with less water, particularly in countries with limited water and land resources. While on a global scale water resources are still ample, serious water shortages are developing in the arid and semi arid regions, as existing water resources are fully exploited. The situation is exacerbated by the declining quality of water and soil resources. Dependency on water for future development has become a critical constraint for development, which threatens to slow down development, endanger food supplies and aggravate rural poverty. Sustainable food production will depend on the judicious use of water resources to meet future

food demands and to address the growing competition for clean water. Water productivity in terms of output per unit of food per m³ of water used needs to be increased in both irrigated and rain fed agriculture substantially: in short: more crop per drop [1]. Drought, salinity, heat and freezing are environmental conditions that cause adverse effects on the growth of plants. Water deficit more than other stresses limits the growth and the productivity of crops [2]. It is known that one of essential nutrients in human consumption oil or fat is applied from the plant and animal source. Oil seed crops are grown throughout of Iran for use as oils [3]. The increasing area of oil seed crop production is an indication of the success of plant breeders and agronomists in developing suitable cultivars and production methods in semi arid region [4]. The lack of oil in Iran has been met by imports that have entailed considerable costs to make up for the lack of oil in Iran,

oil seed production can be increased by growing oil crops in dry land farming or area with water deficit. According to annual precipitation many regions in Iran suffer from water deficit. Canola is one of the best crops for rotation with wheat. High temperature during maturation and ripening is a major source of stress in karaj environments [5]. Without sufficient water to maintain transpiration, leaf temperature can rise above their optimum for metabolism [6]. Seed yield of *Brassica napus*, *B. juncea* and *B. was* increased due to drought stress [7-11]. The effect of drought stress is a function of genotype, intensity and duration of stress, weather, conditions, growth and developmental stages of rape seed [12]. The occurrence time is more important than the water stress intensity [13]. It is known that the most sensitive growth stage to drought stress is seed filling in bean (*phaseolus vulgaris* L.) [14], heading and flowering in wheat (*Triticum aestivum* L.) [15], seed filling in soybean (*Glycine max* L.) [16], flowering and seed filling in pea (*Cicer arietinum* L.) [17], 2-3 weeks after silking in maize (*zea mays* L.) [18], flowering and anthesis in rice (*Oryza sativa* L.) [19]. Other researchers like Angadi *et al.* [20], Mailer and Cornish [21] and Walton and Bowden [22] also reported that during their experiments the post anthesis duration was significantly correlated with the post anthesis rainfall and was negatively correlated with the main daily temperature during seed development.

Rahnema *et al.* [5] reported that the highest rate of yield reduction was occurred by spring irrigation cut off and one spring irrigation treatments in PF which was the late maturity variety. Also the lowest rate of yield reduction was obtained in spring irrigation cut off and one spring irrigation treatments in H308 hybrid respectively. Gunasekera *et al.* [23] reported that rainfall and thus soil moisture are the most important factors affecting crop production in the typical Mediterranean environment. Seed yield is primarily limited by the relatively short duration of soil moisture during the latter phases of reproductive development. Genotypes having great tolerance to water stress, in addition to earliness, generally would have positive effect on improving adaptability and seed yield in such environments. Nielsen [24] reported that water stress during the grain-filling stage resulted in a more rapid loss of leaf area than during other growth stages. Lower yield resulted from fewer branches per plant, pods per branch and smaller seed. Smis *et al.* [25] observed that canola yield in Montana increased with higher availability of water, but had a lower mean oil content. Miller and Cornish [4] determines that oil content fell from 36.9 to

31.4% when high temperature occurred during the post anthesis seed development in canola. Final seed number and seed yield reduction was approved by high temperature effect during reproductive and ripening growth stages. Under dry land conditions, Henry and Macdonald [26] reported that severe drought decreased oil and increased protein content of rape seed. Jensen *et al.* [7] found that under low evaporative demands (2-4 mm day⁻¹) oil and seed yield were not influenced by soil drying. Under high evaporative demands (4-5 mm day⁻¹) oil and seed yields were significantly decreased, but protein content was not. Thompson [27] observed little effect of water stress on seed protein content in soybean. Whereas Hobbs and Muendle [28] reported that drought stress increased protein content. The occurrence time and intensity of drought differ annually in field. Thus, its very important to determine critical stages of oil seed rape crops against drought stress. The growth especially reproductive growth of rape seed is exposed to drought stress in many areas of Iran. For the Iran with high temperature and the shortage of water during stem elongation, flowering and ripening stages still we need to introduce new varieties to farmers which could more adapted to this environment and also to identify the best optimum irrigation level for this region.

MATERIALS AND METHODS

This study was carried out at the experimental farm of the department of agronomy and crop breeding faculty of agriculture and natural resources, science and research branch, Islamic Azad University Karaj Iran 2004-5 and 2005-6. The climatic data of the region are representing in (Table1). The soil has clay loam texture (the values of texture components is missing in the Table 2) and low organic matter (Table2).

The study was established using a split-plot laid out in a RCB Design with four replication. Three water treatments: water stress free (i.e. normal irrigation treatment as control), moderate and high water stress during reproductive growth (from flowering to seed ripening) were as main-plot and Ebonit, Elite and SLM046 cultivars were as sub-plot. Watering of the control, moderate and high water stress treatments occurred when 25, 50 and 75% of AW were depleted, respectively. The amount of water applied was calculated to restore the water to FC, 25 and 50% depletion of AW for control, moderate and high stress treatments, respectively. FC and PWP were measured by pressure plate.

Table 1: Climatic data of experimental farm of I.A.Univ in 2004-5 and 2005-6 (in growth period) *,**

Year and month	Rainfall (mm)	Min temp (°c)	Average temp (°c)	Max temp (°c)	Evapotranspiration (%)
2004-5					
September	3.0	19.8	28.65	37.5	111.7
October	3.5	17.1	25.65	34.2	91.2
November	19.7	14.3	20.40	26.5	80.0
December	95.3	9.0	13.35	17.7	67.1
January	115.6	5.1	9.60	14.1	56.8
February	29.1	5.0	9.75	14.5	61.3
March	13.3	10.1	18.40	26.7	107.1
April	9.4	15.7	22.00	28.3	139.2
May	0.0	26.7	32.75	38.8	361.4
2005-6					
September	11.0	17.1	26.35	35.6	119.2
October	18.9	19.6	27.30	35.0	85.5
November	25.6	12.2	20.60	29.0	75.2
December	91.4	8.1	13.10	18.1	60.2
January	136.1	6.0	9.00	12.0	55.2
February	45.1	4.8	10.40	16.0	59.0
March	10.2	14.4	20.90	27.4	111.0
April	7.2	15.0	22.00	29.0	140.2
May	0.0	30.4	35.10	39.8	389.0

* Taken from the recording of irrigation department in agricultural & natural resource faculty of I.A.Univ., ** (Data recording): meteorological data were collected 300m from the experiment site. Maximum and minimum temperature, rainfall and class A pan evaporation data for the experimental period

Table 2: Result of some chemical and physical analysis of experimental soil*

Depth (cm)	Potassium (ppm)	Phosphor (ppm)	Nitrogen (%)	Organic EC			PH	FC	PWP
				matter (%)	(%)	/cm)			
0-30	171	3.8	0.05	0.49	1.2	7.86	18.75	6.33	
30-60	179	2.8	0.04	0.29	2.19	7.67	17.91	6.36	

*Soil analysis was done at the laboratories of soil science department in I.A. Univ.

Individual plots consisted of 8 rows, 4m long and spaced 30cm apart. Seeds were planted 1 to 1.5cm deep at a rate of 100 seeds m⁻² on 25 September. For all treatments, N:P:K fertilizers applied at a rates of 150:60:50 kg haG¹, respectively. P, K and one-third of N were applied per plant and incorporated. Other two-third of N was split equally at the beginning of the stem elongation and the flowering. All rainfall were excluded by mobile shelter during reproductive growth grass

weeds were controlled by application of gallant-super (Haloxypop r-methyl ester) at 0.6L haG¹ broad-leaf weeds were also hand weeded during the season. Final harvests were carried out at the 30 may. Data collected included acheine yield (obtained by combining the six center rows at each experimental unit), dry matter was determined after drying at 70°C for at least 48 h in an air oven [29, 30]. The following measurements were carried out: biological (above-ground), straw and seed yields, harvest of siliques per plant (with at least on seed), the number index (seed yield divided by biological yield), the number of seed per silique and 1000-seed weight. seed oil and protein content were determined by the Nuclear Magnetic Resonance (NMR) and the Kjeldahl (Protein = 6.25*N) methods, respectively. The experimental data were statistically analyzed for variance using the SAS system [31]. When analysis of variance showed significant treatments effects, Duncan Multiple Range Test was applied to compare the means at P<0.05.

RESULTS

Biological yield decreased 20.7 and 31.2% under moderate and high water stresses compared to the control, respectively. Straw dry matter production also reduced by 21.2 and 30.6%, respectively (Table 3). SLM046 was the most sensitive cultivar to water stress in terms of biological straw and seed yields. Ebonit produced the most seed yield, however seed yield decreased by 19.4 and 32.8% at the moderate high stresses compared to the control, respectively. The number of siliques per plant was the most sensitive yield components to drought stress during reproductive growth. The silique number per plant and seed weight significantly decreased when water stress intensified (Table 3). A little compensation was observed at the moderate water stress by seeds per silique, but there was not under high stress. The number of siliques per plant in SLM 046 was more at the control, but fewer at the moderate and high stresses compared to other cultivars. The number of seeds per silique and 1000-seed weight in Ebonit were significantly higher than other cultivars. The effect of water stress intensity was significant (p = 0.01) on the seed oil and protein contents (Table 3). The effect of water stress was more important on the oil and protein yield than there concentrations. For example, the oil concentration decreased only by 0.39 and 2.16% in the moderate and high water stresses compared to the

Table 3: Bennial mean comparison of biological(BY)straw(StY)and seed(SY)yields, harvest index(HI),the number of siliques per plant(Si/Pl),the number of seeds per silique (S/Si),seed weight (SW),oil content(OC),protein content(PC) and oil yield (OY)in different water stress intensities, cultivars and their interaction (for the field experiment)

Treatment	BY(g mG ²)	StY(g mG ²)	SY(g mG ²)	HI(%)	Si/Pl	S/Si	SW(mg)	OC(%)	PC(%)	OY(g mG ²)
Control(w1)	1125a	807a	318a	28.1a	103a	20.7a	3.37a	48.4a	20.3b	154.0a
Mod. stress(w2)	894b	638b	258b	28.8a	78b	20.9a	3.17b	48.0a	20.7b	124.0b
High stress(w3)	775c	561c	214c	27.6b	63c	19.8a	3.16b	46.3b	22.9a	99.1c
LSD 5%	77	45	19	1.46	6	1.59	0.15	0.71	1.28	6.53
Ebonit	935a	614b	321a	34.3a	77b	23.2a	3.73a	48.3a	19.6b	155.6a
Elite	932a	694a	238b	25.5b	84a	18.9b	2.99b	46.3a	22.2a	110.2b
SLM046	929b	699a	230b	24.7b	84a	19.3b	2.98b	48.2a	22.2a	110.9b
LSD 5%	70	41	14	1.14	6	1.17	0.17	0.55	0.7	5.32
V1*w1	1106a	731b	375a	33.9a	92c	23.5a	3.88a	48.0a	19.1c	180.0a
V1*w2	905b	589de	316b	34.9a	77de	23.2a	3.59b	48.7a	18.8c	153.9b
V1*w3	794cd	520e	274d	34.5a	61fg	22.8a	3.71ab	47.5b	21.0b	130.1c
V2*w1	1117a	831a	286cd	25.6b	104b	19.5bc	3.08cd	47.2b	20.9b	135.0c
V2*w2	912b	677bc	235e	25.7b	81d	19.3bc	2.99cd	46.7b	21.9b	110.2d
V2*w3	789cd	593de	196f	24.8b	69ef	17.9c	2.91cd	44.8c	23.6a	88.0e
V3*w1	1181a	883a	298c	25.2b	114a	19.0bc	3.14c	49.3a	21.0b	146.9b
V3*w2	867bc	646cd	221e	25.5b	78d	20.2b	2.93cd	48.7a	21.4b	108.0d
V3*w3	741d	569de	172g	23.2c	60g	18.7bc	2.85cd	46.5b	24.2a	80.0e
LSD 5%	86	73	18	1.36	8	1.44	0.21	0.95	1.21	9.21

Mean followed by the same letter(s) in each column (between to horizontal lines)are not significantly different (Duncan 5%)

control, but the oil yield decreased by 20and 35.6% respectively the protein yield also decreased despite increased protein concentration.

DISCUSSION

First, flowering, silique development were the critical stages of Canola to water stress. The seed yield reduction due to water stress during flowering (S2) was associated with the reduction of the silique number per plant (26.5%) and the seed number per silique (9.9%). The seed yield reduction at S1 and S3 treatments were also associated mostly with the reduction of the silique per plant. Water stress during seed development (S4) reduced seed yield via reduction of seed weight. Since, water stress during seed development did not affect on the sink size (seeds per plant), decreased source capacity led to reduction of seed weight. Richard and Thurling [32] observed that some cultivars were more sensitive at flowering and others were at silique development. Mingeau [33] demonstrated a critical period from anthesis to anthesis +2 weeks, that seed yield was reduced by (20%) due to water stress during this period. Champolivier and Merrien [34] reported that the most sensitive period of *B.napus* to water stress was between flowering and silique development. Water stress during vegetative or early

reproductive stages of soybean usually reduces yield by reduction of seed number per unit area [13], 1 while stress during seed filling reduces seed size [16, 35, 36]. In a field experiment on *B.napus* and *B.juncea* in west Australia, Gunasekara *at al.* [23] observed that the mean biological yield was decreased by 17.9 and 32.1% and the mean seed yield was decreased by 18.5 and 38.7% by moderate and high water stresses during reproductive growth compared to the control, respectively The number of silique per plant was the most sensitive yield components to water stress during reproductive growth in both pot and field experiments. In Canola, Mendham *et al.* [37] have argued that Canola breeders should be aiming to produce plants with fewer pods but with a higher potential number of seeds per pod as this maximizes seed survival and hence increases seed number per unit area. A similar ideotype has been suggested for both Canola and mustard [38, 39]. There was not the compensation effect between yield components under long-term water stress in the pot and the field experiments. Clarke and Simpson [40] did not observe any compensation between the number of siliques and seeds per silique, too. Kumar *et al.* [41] demonstrated increased 1000-seed weight (a compensation effect) following water stress and reduced the seeds per plant. Straw and seed yields were similarly influenced at S2, S3 and S4 treatments,

consequently harvest indices did not differ compared to the control, harvest index was higher at S1 treatment, because water stress during stem elongation was more affected straw dry matter production than seed yield. The reduction of vegetative dry matter is due to reduction of leaf area and photosynthesis rate [11]. Long-term water stress during reproductive growth decreased harvest index in the pot experiment, but did not affect in the field experiment. Ali *et al.* [42] observed an increase in harvest index following drought stress, but Wright *et al.* [39] obtained reduction of harvest index..

CONCLUSIONS

The objective of this study was to test the hypothesis that although annual precipitation in Iran has variation in period, time and content but to decrease the oil seed import to Iran is this possible that use the new region with water deficit. Also could be produced oil seed by canola in this area. In addition what is the effect of water deficit in different stages of vegetative and reproductive on yield components, seed yield, seed oil and protein contents. It is concluded from the present study that water stress during reproductive growth of canola mainly decreased seed yield by reduction of the silique number per plant. The number of seeds per silique less change than the silique per plant. Therefore, selection or breeding of genotypes with high seeds per silique seems better under water deficit conditions. This characteristic leads to higher seed yield with higher seed yield stability under drought stress. Even though seed weight is usually depend on genotype, heavier individual seed weight is also a good characteristic.

REFERENCES

1. FAO, 2000. Crops and Drops, Land and Water Development Division, FAO, Rome, Italy, pp: 24.
2. Yamaguchi-Shinozaki, K., M. Kasuga, Q. Liu, K. Nakashima, Y. Sakuma and Habe, 2002. Biological mechanisms of drought stress response. JIRCAS Working Report, pp: 1-8.
3. Nabipour, M.M. Meskarbasheh and H. Yousefpour, 2007. The effect of water deficit on yield and yield component of safflower (*Carthamus tictorius* L.). Pak. J. Biol. Sci., 10: 421-426.
4. Miller, P., D. Baltensperger, G. Clayton, A. Johnson, G. Lafond, B. McConkey, B. Schatz and J. Starica, 2002. Pulse crop adaptation and impact across the northern Great Plains. Agron. J., 94: 261-272.
5. Rahnema and A.M. Bakhshande, 2006. Determination of optimum irrigation level and compatible canola varieties in the Mediterranean environment. Asian J. Plant Sci., 5: 543-546.
6. Mahan, J.R. and D.R. Upchurch, 1988. Maintenance of constant leaf temperature by plants. I. Hypothesis Limited homeothermy. Environ. Exp. Bot., 28: 351-357.
7. Jensen, C.R., V.O. Mogensen, J.K. fieldsen and J.H. Thage, 1996. Seed glucosinolate, oil and protein contents of field-grown rape (*Brassica napus* L.) affected by soil drying and evaporative demand. Field Crops Res., 47: 93-105.
8. Kumar, A. and D.P. Singh, 1998. Use of physiological indices as a screening technique for drought tolerance in oilseed *Brassica* species. Ann. Bot., 81: 413-420.
9. Sharma, D.K. and A. Kumar, 1989. Effect of irrigation on growth analysis, yield and use water in Indian mustard (*Brassica juncea* L.). Indian J. Agric. Sci., 59: 162-165.
10. Sharma, D.K., 1992. Physiological analysis of yield variations in mustard varieties under water stress and non water stress conditions. Ann. Agric. Res., 13: 174-176.
11. Wright, G.C., C.J. Smith and M.R. Woodroffe, 1988. The effect of irrigation and nitrogen fertilizer on rapeseed (*Brassica napus* L.) production in south-eastern Australia. Irrig. Sci., 9: 1-13.
12. Robertson, M.J. and J.F. Holland, 2004. Production risk of canola in the semi-arid subtropics of Australia. Aust. J. Agric. Res., 55: 525-538.
13. Korte, L.L., J.H. Williams, J.E. Specht and R.C. Sorenson, 1983. Irrigation of soybean genotypes during reproductive ontogeny: II. Yield component responses. Crop Sci., 23: 528-533.
14. Nielsen, D.C. and N.O. Nelson, 1998. Black bean sensitivity to water stress at various growth stages. Crop Sci., 38: 422-427.
15. Moustafa, M.A., L. Boersma and W.E. Kronstad, 1996. Response of four spring wheat cultivars to drought stress. Crop Sci., 36: 982-986.
16. Brevedan, R.E. and D.B. Egli, 2003. Short periods of water stress during seed filling, leaf senescence and yield of soybean. Crop Sci., 43: 2083-2088.
17. Singh, P., 1991. Influence of water deficits on phenology, growth and dry matter allocation in chickpea (*Cicer arietinum* L.). Field Crops Res., 28: 1-15.
18. Grant, R.F., B.S. Jackson, J.R. Kiniry and G.F. Arkin, 1989. Water deficit timing effects on yield components in maize. Agron. J., 81: 61-65.

19. Lilley, J.M. and S. Fukai, 1994. Effect of timing and severity of water deficit on four rice cultivars. III. Phenological development, crop growth and grain yield. *Field Crop Res.*, 37: 225-234.
20. Angadi, S.V., H.W. Cutforth, B.G. McConkey and Y. Gan, 2003. Yield adjustment by canola growth at different plant populations under semiarid conditions. *Crop Sci.*, 43: 1358-1366.
21. Mailer, R.J. and P.S. Cornish, 1987. Effects of water stress on glicosinolate and oil contents in the rape (*Brassica napus* L.) and turnip rape (*B. rapa* L.). *Aust. J. Exp. Agric.*, 27: 707-711.
22. Walton, G., P. Si and B. Boden, 1997. Environmental impact on canola yield and oil. www.Canola-ra/canola/ca7.htm.
23. Gunasekara, C.P., L.D. Martin, R.J. French, K.H.M. Siddique and G.H. Walton, 2003. Effects of water stress on water relations and yield of Indian mustard (*Brassica juncea* L.) and canola (*B. napus* L.). Proceedings of the 11 th Australian Agronomy Conference, Geelong, Australia.
24. Nielsen, D.C., 1997. Water use and yield of canola under dry land conditions in the central great planins. *Agriculture*, 10: 307-313.
25. Smis, J.R., D.J. Solum, D.M. Wichman, G.D. Kushnk and L.E. Welty *et al.*, 1993. Canola variety yield trials. *Montana Agro. Res.*, 10: 15-20.
26. Henry, J.L. and K.B. MacDonald, 1978. The effects of soil and fertilizer nitrogen and moisture stress on yield, oil and protein concentration of rape. *Can. J. Soil Sci.*, 58: 303-310.
27. Thompson, J.A., 1978. Effect of irrigation interval and plant population on growth, yield and water use of soybean in semi-arid environment. *Aust. J. Exp. Agric. Husb.*, 18: 276-281.
28. Hobbs, E.H. and H.H. Muendel, 1983. Water requirements of irrigation soybeans in southern Alberta. *Can. J. Plant Sci.*, 63: 855-860.
29. Schuurman, J.J. and M.A.J. Goedewaagen, 1971. *Methods for Examination of Root Systems and Roots*. 2nd Ed., Wageningen: Pudoc.
30. Veli, S., Y. Kirtok, S. Duzenli, S. Tukul and M. Klinec, 1994. Evaluation of salinity stress on germination characteristics and seedling growth of 3 bread wheat (*T. aestivum* L.). *Tarla Bitkileri Kong Agronomy Bildirileri, Bornova-Izmir. Cilt.*, 1: 57-61.
31. SAS Institute Inc., 1997. *SAS/STAT Users Guide*, version 6, 4th Ed., SAS Institute., Cary, NC, USA.
32. Richards, R.A. and N. Thurling, 1978. Variation between and within species of rape seed (*Brassica campestris* and *B. napus*) in response to drought stress. II. Growth and development under natural drought stress. *Aust. J. Agric. Res.*, 29: 479-490.
33. Mingeau, M., 1974. Comportement du colza de printemps a la secheresse. *Inf. Tech. CETIOM*, 36: 1-11.
34. Champolivier, L. and A. Merrien, 1996. Effects of water stress applied at different growth stages to *Brassica napus* L. var. *oliefera* on yield, yield components and seed quality. *Europ. J. Agron.*, 5: 153-160.
35. Meckel, L., D.B. Egli, R.E. Phillips, D. Radoliffe and J.E. Leggett, 1984. Effect of moisture stress on seed growth soybean. *Agron. J.*, 76: 647-650.
36. De-Souza, P.I., D.B. Egli and W.P. Bruening, 1997. Water stress during seed filling and leaf senescence in soybean. *Agron. J.*, 89: 807-812.
37. Mendham, N.J., J. Russel and G.C. Buzza, 1984. The contribution of seed survival to yield in new Australian cultivars of oil seed rape (*Brassica napus* L.). *J. Agric. Sci. Camb.*, 103: 303-316.
38. Bhargava, S.C. and D.P.S. Tomar, 1990. Physiological contribution for increased yields in rapeseed mustard. In: *proceedings of the International congress of plant physiology*. 15-20 feb. 1987, New Delhi, pp: 354-360.
39. Wright, P.R., J.M. Morgan, R.S. Jossop and A. Cass, 1995. Comparative adaptation of canola (*Brassica napus* L.) and Indian mustard (*B. juncea* L.) to soil water deficit. *Field Crops Res.*, 42: 1-13.
40. Clarke, J. and G.M. Simpson, 1978. Influence of irrigation and seeding rates on yield and yield components of *Brassica napus* cv. Tower. *Can. J. Plant Sci.*, 58: 731-737.
41. Kumar, A., Elston and P. Singh, 1994. Leaf area growth to two *Brassica* species in response to water stress. *Field Crops Res.*, 8: 594-602.
42. Ali, M.A., I. Ohlsson and H. svensk, 1988. Drought stress response in rapeseed (*Brassica Juncea* L. and *B. napus* L.). growth, yield and yield components. *Agric. Hortic. Genet.*, 46: 16-48.

Differential Response and Signal Transduction Due to Cold Shock and Oxidative Stress in *Bacillus simplex* TWW-04

¹O.M. Gomaa and ²O.A. Momtaz

¹National Center for Radiation Research and Technology (NCRRT), P.O. Box 29, Nasr City, Cairo, Egypt

²Agricultural Genetic Engineering Research Institute (AGERI), 9 Gama St. Giza, Cairo, Egypt

Abstract: The accumulation of stress proteins in the cytoplasm, periplasm or membrane of bacteria is considered an initial signal which induces the expression of genes involved in defense, recovery and stress acclimation. Whole cell protein profile for *Bacillus simplex* TWW-04 showed a gradual increase in expressed proteins by increasing the hydrogen peroxide concentration, this was different from the heat shock proteins induced at 60°C and were closer to the cold-shock proteins expressed at 4°C, a marked increase in band number and intensity varied by the increase in hydrogen peroxide concentrations. The bacterial count and total protein content showed a decrease in cold-shock cultures, hydrogen peroxide (150 mM) and gamma (0.5 KGy) exposed cultures after 4 h. The colony forming ability for cultures exposed to cold-shock, hydrogen peroxide and gamma radiation showed no change after 4 h, but after 16 h of incubation, only the hydrogen peroxide culture failed to show any colony forming ability. The growth pattern followed by absorbance at O.D₆₀₀ after stress exposure showed different response for each culture. The level of membrane lipid unsaturation was measured through FTIR, the spectra showed a close pattern for both the cold-shock and oxidative stress exposed cultures at the characteristic wave number for C=C. It is therefore concluded that the response of *Bacillus simplex* TWW-04 during oxidative stress is close to that exhibited during cold-shock; the role is similar in protecting the cell membrane from damage during hydroxyl attack by increasing the ratio of unsaturated fatty acids to saturated fatty acids in the membrane lipids.

Key words: *Bacillus simplex* . oxidative stress . protein profile . stress response . lipid desaturation

INTRODUCTION

Biological membranes are essential for the cell integrity, providing a barrier between the inside and outside environments for the cell [1]. These barriers act as support to different proteins which are involved in an array of different cell functions such as energy transduction, signal transduction, solute transport, DNA replication, cell-cell recognition, protein targeting and trafficking [2]. A number of studies suggest that membranes can sense extreme environmental changes such as cold-shock, heat stress or the presence of oxidants in the media [3-5].

Bacteria respond to such extreme changes in environmental conditions by inducing unique groups of proteins, each according to the type of stress, there are also another set of commonly synthesized proteins, regardless of the type of stress induced. Approximately 30 common proteins shared between vegetative proteins, stress and starvation in *Bacillus subtilis* [6]. Another common adaptation response among all

bacteria is the adjustment of membrane lipid composition at low temperatures [7], this change is also reported due to oxidative stress [8]. *Bacillus simplex* TWW-04 was studied for its tolerance to hydrogen peroxide and low temperature, its adaptation was due to the production of a number of counteracting enzymes [9], the study of the cell membrane showed that its fatty acid composition was affected under oxidative stress conditions [10]. It was assumed that the membrane lipoproteins are the major component responsible for loss of colony forming ability. Previous studies show that membranes have the ability to sense external changes and as a consequence, changes occur in its physical state and microdomain organization, sending signals which activate transcription [3, 7]. Therefore, it seemed likely that bacteria sensing the environmental changes could start sending signals for transcription of different counteracting enzymes via an initial change in the membrane's biophysical state. The pattern is assumed to be close to that for cold-shock response since both oxidative stress (via the addition of hydrogen

peroxide) and cold-shock are considered initial signal transducers in bacteria [8]. The increase of unsaturated fatty acids in the cell membrane is a general response in some thermotolerant strains exposed to one or a combination of stresses especially oxidative stress [11]. Besides adaptation to external stress, the alteration in membrane fatty acid desaturation is thought also to be for maintenance of membrane functionality at low temperature [12].

In the present study, *Bacillus simplex* TWW-04 is exposed to sublethal oxidative stress and compared to cold-shock in order to compare the regulation response of this strain to both stresses. The protein profile, protein content, colony count, colony forming ability and growth pattern for the different cultures are studied. The degree of unsaturation for both cold-shock and oxidative stress of membrane fatty acids under the same conditions is examined by FTIR.

MATERIALS AND METHODS

Microorganism and culture conditions: *Bacillus simplex* TWW-04 obtained from a previous study [13] was used to inoculate LB media, static cultures were incubated at 30°C for 24 h. Two % of preculture inoculum (vol/vol) was used to inoculate flasks containing 50 ml LB media, H₂O₂ (30%) was added in different concentrations (50, 100 and 150 mM) after sterilization. Cultures were incubated for 120 minutes at 30°C and centrifuged rapidly to stop the action of hydroxyl radicals formed. For +ve control, cultures were exposed to cold shock, cultures were transferred to 4°C. For -ve control, cultures were exposed to 60°C. For culture growth under different stresses, the cultures O.D was measured at 600 nm for 60, 120, 240 and 300 min.

Irradiation process: Gamma irradiation was used in order to study another form of sublethal oxidative stress and its effect on the bacterial cells. A 50 ml sample of the mid-exponential liquid culture was subjected to gamma radiation at National Center for Radiation Research & Technology (NCRRT) using ⁶⁰Co source with dose rate 1.85 Gy/sec. The irradiation dose was 0.5 KGy.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE): Whole cell cultures were centrifuged at 3000 rpm for 15 minutes after each treatment, Phosphate Buffer Saline (PBS) (pH 7.2) was used for protein extraction. SDS-PAGE was performed for whole cell protein extracts at room temperature with 10% gels and Tris-glycine buffer (pH 8) at 125 V for 90 minutes to obtain the molecular weight of the enzymes,

protein bands were stained by 0.05% coomassie brilliant blue R-250. Molecular weights were compared to a low/medium protein marker (Bio-Rad). Protein gel documentation was performed using Alphatec 2200 software for protein band analysis.

Colony Forming Ability (CFA), colony count and total protein: For the detection of colony forming ability, 10 µl was spotted using a sterile tip onto LB agar plates at the chosen time. Photos were taken after 24 hr incubation at 30°C.

To measure the number of colonies, 100 µl aliquots of 10⁻⁹ time diluted cultures were spread onto LB-agar plates and incubated for 24 h at 30°C.

Protein concentrations were determined by the method of Lowry *et al.* [13] using bovine serum albumin as a standard using Shimadzu UV 2100 spectrophotometer.

FTIR analysis of membrane lipids: The analysis was performed on cultures exposed to cold-shock and hydrogen peroxide and were compared to that of a control culture. Cultures were centrifuged at 3000 rpm for 15 minutes to collect the cells, resuspended in phosphate buffer pH 7.2, ultra centrifuged (Sorvall ultra 80) at 100,000 rpm at 4°C for 10 minutes, the supernatant decanted and the pellets freeze-dried using Modulyo, Edwards Freeze-Drier, the dried pellets were homogenized and prepared for analysis using the FTIR (Unicam mattsom 1000).

RESULTS AND DISCUSSION

Microorganisms adapt to different environmental stresses, primarily by producing a number of stress proteins, some are general and others are specified according to the type of stress [6]. In facing stress, *Bacillus* cells have developed complex adaptational network, inside of which the induction of general or unspecific stress proteins seems crucial to its survival [14]. A set of about 50 proteins, which are induced by many environmental stimuli, is one of the most drastic responses of the *B. subtilis* cell to the transition from a growing to a non-growing state [15]. Accumulation of heat stress proteins (HSPs), which are termed chaperones, or the appearance of cold-shock proteins enable the cells to accommodate to such variant temperatures which could halt growth and damage many physiological parameters. In the current study, it is evident that the resulting protein profile reflects a change in protein synthesis related to the different stresses induced. Visual examination of Fig. 1 shows the SDS-PAGE for the whole cell proteins exposed to different types of stress, the increase in band intensity shows an increase in protein expression in cells with the

Table 1: Represents the data obtained from the analysis of the gel, data show the number of peaks, area and molecular weight for each distinct band

Lane	Peak	Dist	Width	Height	Area	%	MW
M	1 mws	73	14	213	3132	10.9	192.77
M	2 mws	92	5	226	1282	4.5	117.90
M	3 mws	134	19	241	4700	16.3	99.26
M	4 mws	231	26	242	6350	22.0	54.15
M	5 mws	295	22	241	5376	18.7	37.78
M	6 mws	330	12	242	2964	10.3	29.46
M	7 mws	436	19	225	4083	14.2	20.20
M	8 mws	453	6	77	913	3.2	7.44
-ve control	1	134	28	207	5844	44.0	116.42
-ve control	2	231	36	208	7443	56.0	79.26
+ve control	1	131	20	218	4303	27.8	117.57
+ve control	2	231	52	225	11165	72.2	79.26
1	1	131	22	199	4606	29.9	117.57
1	2	231	53	202	10775	70.1	79.26
2	1	118	13	191	2369	5.6	122.55
2	2	150	29	185	5454	12.9	110.29
2	3	340	163	232	34294	81.4	37.49
3	1	124	19	216	4043	23.7	120.26
3	2	147	12	201	2610	15.3	111.44
3	3	177	7	192	1429	8.4	99.95
3	4	224	41	213	8969	52.6	81.94

(M) Denotes for the marker (low/medium), (-ve control)heat shock, (+ve control) cold-shock, (1) hydrogen peroxide 50 mM, (2) hydrogen peroxide 100 mM, (3) hydrogen peroxide 150 mM

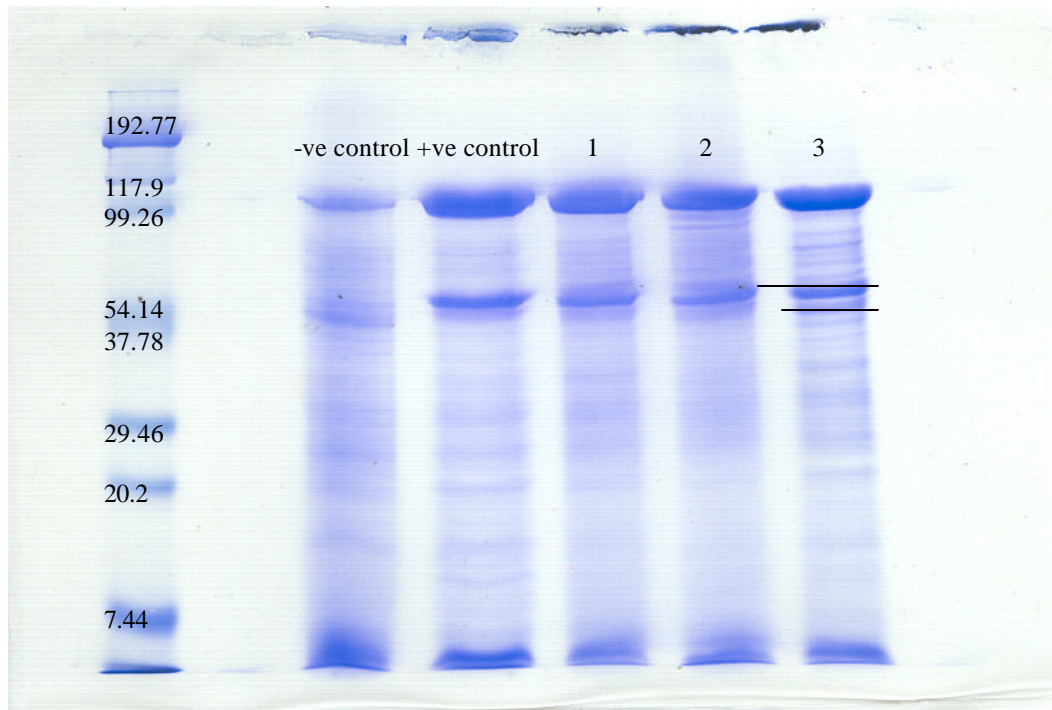


Fig. 1: SDS-PAGE for total cell protein of *Bacillus simplex* TW-04 exposed to 60°C (-ve control), 4°C (+ve control), hydrogen peroxide 50 mM (1), 100 mM (2) and 150 mM (3)

increase in hydrogen peroxide concentration but doesn't show a pattern resembling HSPs but rather close to the +ve control which is the cold-shock induced proteins. This is logical as this strain was known to tolerate to grow at extremely low temperatures and unable to grow

at 40°C [16]. The computer analysis of the gel using Alphatec 2200 reveals in depth data. The number of bands and their areas differ by the stress induced; also there is an increase which is relevant to the concentration of hydrogen peroxide indicating a

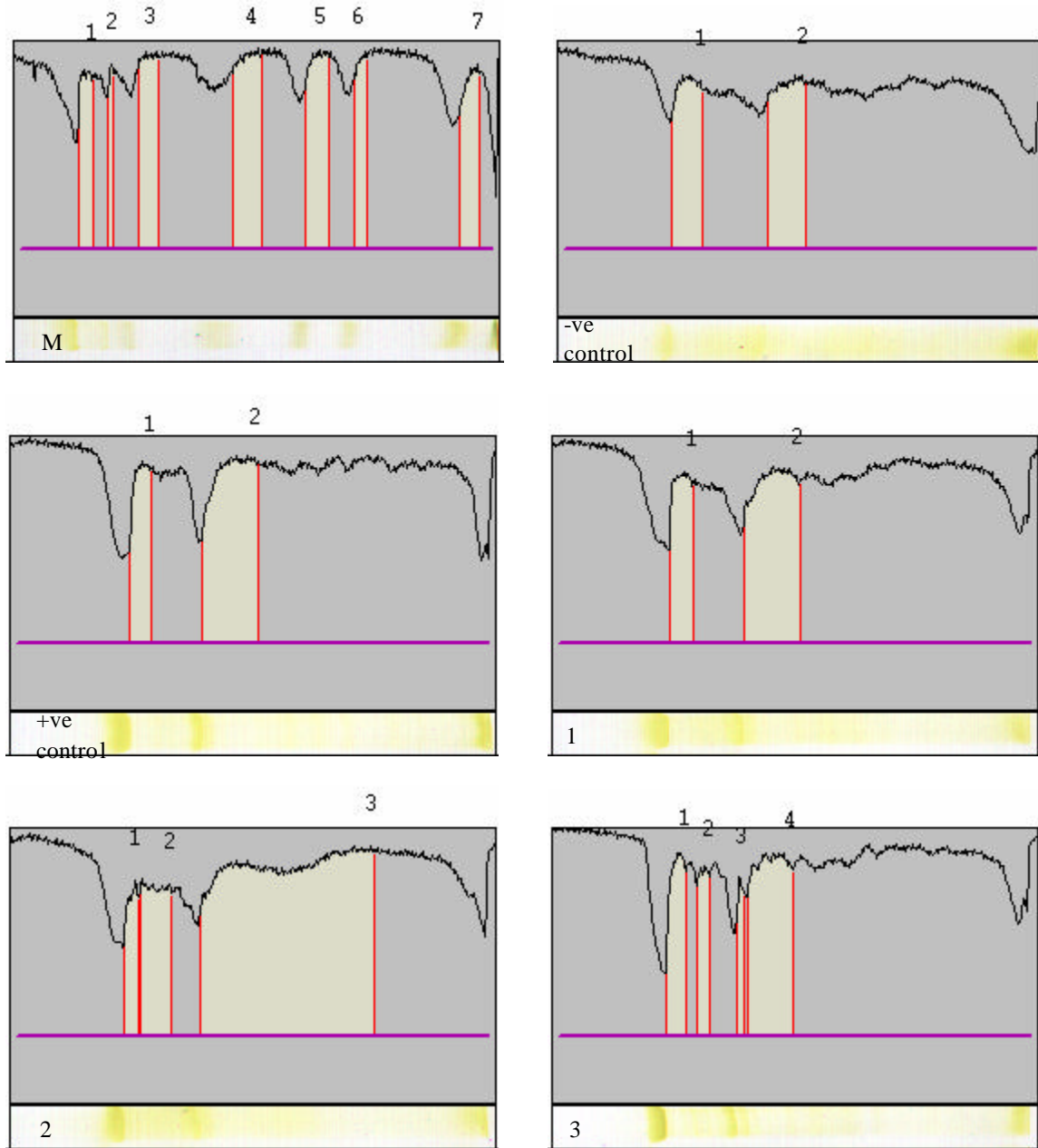
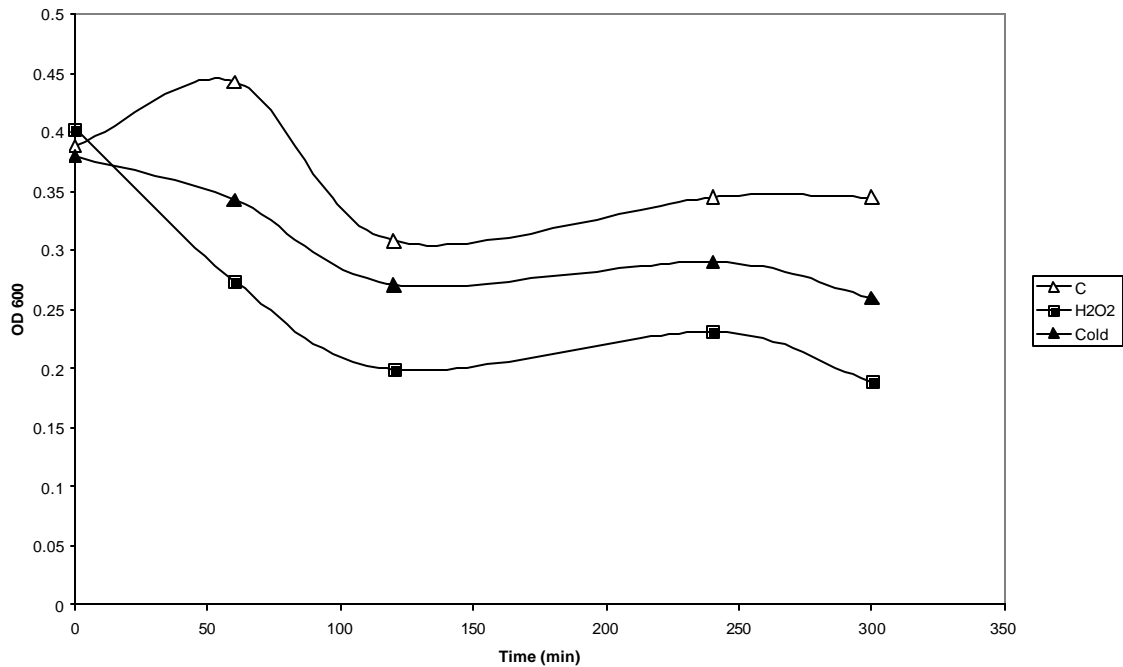


Fig. 2: Analysis of the obtained bands by the protein analysis software for the number and area for each peak obtained for every lane (M) marker, -ve control, +ve control, (1) hydrogen peroxide 50 mM, (2) hydrogen peroxide 100 mM, (3) hydrogen peroxide 150 mM

Table 2: CFU and protein content for cells subjected to different treatments in comparison to control cells after 4 h

Treatment	CFU ml ⁻¹	Protein (mg ml ⁻¹)
Control	8x10 ⁷	25.70
Cold-shock	23x10 ⁵	22.13
Hydrogen peroxide	12x10 ³	16.65
Gamma-irradiation	18x10 ³	19.20

relatively increased level of protein synthesis which is relevant to the level of stress induction (Fig. 2). Table 1 represents these changes. It is evident that there are two distinctive bands obtained at MW 117 and 79 KDa in -ve, +ve control and culture exposed to 50 mM hydrogen peroxide, the areas of which were the same for cold-shock and 50 mM hydrogen peroxide cultures. Another band appeared when the culture was exposed to 100 mM and a fourth band appeared at 150 mM



ig. 3: Growth of *Bacillus simplex* TWW-04 in LB media with no stress induced (Δ), after cold-shock at 4°C (\blacktriangle) and after the addition of 150 mM hydrogen peroxide (\blacksquare). The cultures were grown on LB at 30°C until OD₆₀₀ of 0.38-0.4 was reached before inducing cold-shock or oxidative stress

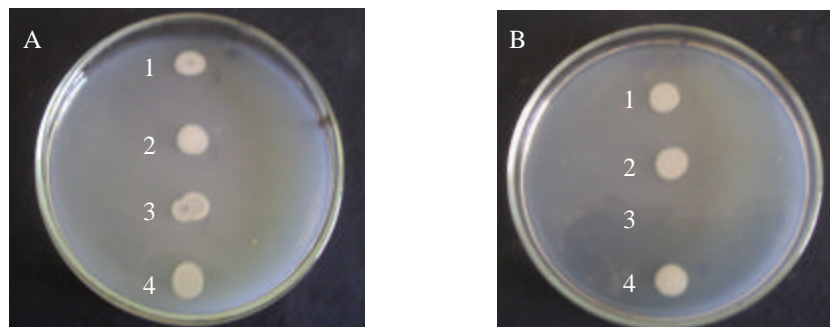


Fig. 4: LB plates representing the colony forming ability for the different cultures after exposure to the stress for 4 hours (A) and 16 hours (B). The samples represent (1) control culture, (2) cold-shock, (3) oxidative stress by the addition of 150 mM hydrogen peroxide, (4) 0.5 KGy gamma radiation

hydrogen peroxide. The table shows the distinct variation in the bands intensity. It is not strange that the pattern of protein synthesis might be similar when cultures are exposed to two different abiotic stresses, there are some regulons which are generally induced in bacteria exposed to parquat, heat, hydrogen peroxide and salt stress in *Bacillus subtilis*, sometimes overlapping occurs among two or more stress conditions, the same occurred when cells were tested for starvation [6].

To assess the level of response of cells to the stresses, a number of parameters were tested. The colony count of the cultures subjected to low doses of

hydrogen peroxide, gamma radiation or cold-shock revealed an obvious decrease in colony forming units after 4 hours of incubation, except for hydrogen peroxide exposed cultures, all cultures showed maintenance for their colony forming ability even after 16 hours of exposure (Fig. 3 and Table 2). This experiment was conducted to ensure the visual response of the cell viability under the different stresses used in this study, the results are in agreement with previous studies for the same strain [5, 16].

The whole cell protein levels (Table 2) were affected by the oxidative stress, indicating an interaction with proteins which could be attributed to an

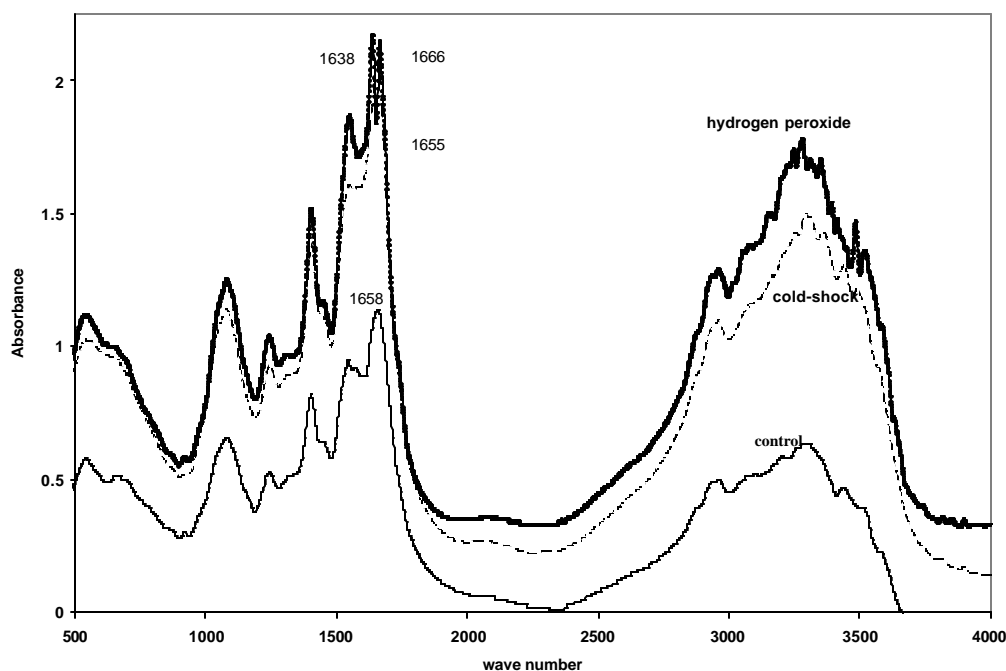


Fig. 5: FTIR for control cultures (no stress), cultures exposed to cold-shock and cultures exposed to oxidative stress by the addition of hydrogen peroxide (150 mM)

Table 3: Wave number and intensity for the peaks of cold-shock and hydrogen peroxide exposed cultures compared to the control

Culture	Wave number	Intensity
Control	1658.92	1.14
	-	-
Cold-shock	1088.34	0.66
	1655.68	2.07
Hydrogen peroxide	-	-
	1080.14	1.14
	1638.86	2.17
	1666.77	2.15
	1080.76	1.25

interaction between hydroxyl radicals formed via the addition of hydrogen peroxide and gamma radiation which interacts directly with the cells. Cold-shock cultures showed a slight decrease in total protein concentrations. Although the three cultures showed similar growth pattern, yet they didn't exhibit the same growth rate, hydrogen peroxide exposed cultures experienced the slower growth, while cold-shock cultures were intermediate between control cultures and hydrogen peroxide (Fig. 4). The results are in accordance with Weber *et al.* [12] and Gomaa and Momtaz [16]. All previous experiments didn't precisely reveal the exact similarity between cell response to cold-shock and oxidative stress. Therefore, it was essential to study a relevant parameter which could explain the resistance of *Bacillus simplex* TWW-04 to

both cold-shock and oxidative stress and highlight the cell response to these stresses. It is known that low temperature causes changes in membrane lipid composition, these changes could be alteration of fatty acid types, altered lipid classes, changes in lipid to protein ratios or increase in fatty acyl unsaturation [17].

Figure 5 represents the spectrum using FTIR of cell membrane to show the degree of lipid unsaturation exhibited at approximately 1650 (characteristic for the C=C), there is an obvious shift in the wave number for the cultures exposed to stress compared to the control culture. Also, the intensity of unsaturation increases by the exposure to both cold shock and oxidative stress, compared to the control sample (2.07 and 2.17 for the stress exposed cultures compared to 1.14 for the control culture), there is splitting in the peak at 1638.86 and 1666.77 for the hydrogen peroxide exposed culture with an increase in the intensity. There is an obvious stretching in the C-C bond at approximately 1080 from 1.14 for the cold-shock culture and 1.25 for the hydrogen peroxide exposed culture compared to 0.66 for the control culture (Table 3).

The degree of fatty acyl desaturation of membrane lipids is considered to be a critical determinant in membrane fluidity [18], this membrane fluidity is a trigger for membrane remodeling and aids in bacterial tolerance to heat in *E. coli* [4]. The relation between fatty acyl desaturation and bacterial adaptation was reported for a number of strains exposed to heat, salinity, cold or oxidative damage [4, 11, 18, 19]. This

implies that membrane lipids play an essential role in microbial adaptation under different environmental conditions.

It is our conclusion that *Bacillus simplex* TWW-04 responds to oxidative stress through tight regulation of the lipid composition of the cell in a similar mechanism to low thermal adaptation, this mechanism is believed to be designed to ameliorate the external changes on the physical state of the cell membrane through desaturation of fatty acids of their membrane lipids. Cold shock adaptation was found to be through *des* gene encoding $\Delta 5$ desaturase, this enzyme is responsible for increasing the ratio of unsaturated fatty acids (UFA) to saturated fatty acids (SFA) [20], it is present constitutively in all prokaryotes and eukaryotes in response to decrease in temperature. It was also reported that the acyl chain desaturase was proved to be an integral membrane protein in yeast [18], this proves that the first reaction of microbial cells upon exposure to stress is a change in the degree of acyl chain desaturation of membrane lipids, this occurs before the cell sends signals to produce a set of specific proteins to counteract the damage exerted.

It is therefore speculated that the *Bacillus* under study might use the same approach since it tolerates cold-shocks as well as high concentrations of hydrogen peroxide. The identification of the gene responsible for this adaptation is currently under investigation in our laboratory.

REFERENCES

1. Pedersen, U.R., C. Leidy, P. Westh and G.H. Peters, 2006. The effect of calcium on the properties of charged phospholipids bilayers. *Biochimica et Biophysica Acta*, 1758: 573-582.
2. Edidin, M., 2003. Lipids on the frontier: A century of cell-membrane bilayers. *Nat. Rev. Mol. Cell Biol.*, 4: 414-418.
3. Aguilar, P.S., A.M. Hernandez-Arriaga, L.E. Cybulski, A.C. Erazo and D.de Mendoza, 2001. Molecular basis of thermosensing: A two-component signal transduction thermometer in *Bacillus subtilis*. *The EMBO J.*, 20: 1681-1691.
4. Shigapova, N., Z. Torok, G. Balogh, P. Goloubinoff, L. Vigh and I. Horvath, 2005. Membrane fluidization triggers membrane remodeling which affects the thermotolerance in *Escherichia coli*. *Biochem. Biophys. Res. Comm.*, 328: 1216-1223.
5. Gomaa, O. and N. Selim, 2007. Detection of cell viability by dielectric properties for *Bacillus* sp. *Egypt. J. Rad. Sci. Appl.*, 20: 603-618.
6. Tam, L.T, H. Antelmann, C. Eymann, D. Albrecht, J. Bernhardt and M. Hecker, 2006. Proteome signatures for stress and starvation in *Bacillus subtilis* as revealed by a 2-D gel image color coding approach. *Proteomics*, 6: 4565-4585.
7. Vigh, L., B. Maresca and J.L. Harwood, 1998. Does the membrane's physical state control the expression of heat shock and other genes? *Trends Biochem. Sci.*, 23: 369-374.
8. Bienert, G.P, J.K. Schjoerring and T.P. Jahn, 2006. Membrane transport of hydrogen peroxide. *Biochimica et Biophysica Acta*, 1758: 994-1003.
9. Gomaa, O. and K.Sh. Azab, 2007. Resistance to hydrogen peroxide in textile wastewater *Bacillus* sp. *Intl. J. Agric. Biol.*, 9: 347-351.
10. Gomaa, O. and Kh.Sh. Azab, 2007. Role of calcium carbonate in protecting the colony forming ability of *Bacillus simplex* TWW-04 exposed to oxidative stress. *Adv. Biol. Res.*, (In Press).
11. Guerzoni, M.E., R. Lanciotti and P.S. Cocconcelli, 2001. Alteration in cellular fatty acid composition as a response to salt, acid, oxidative and thermal stresses in *Lactobacillus helveticus*.
12. Weber, M.H.W., W. Klein, L. Muller, U.M. Niess and M.A. Marahiel, 2001. Role of the *Bacillus subtilis* fatty acid desaturase in membrane adaptation during cold shock. *Mol. Microbiol.*, 39: 1321-1329.
13. Gomaa, O. and O. Momtaz, 2006 Characterization of the hydrogen peroxide tolerating bacteria "*Bacillus maroccanus*" type strain isolated from textile waste water. *Arab. J. Biotechnol.*, 9: 83-94.
14. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1957. Protein measurement with phenol-folin reagent. *J. Biol. Chem.*, 193: 265-275.
15. Hecker, M., W. Schumann and U. Volker, 1996. Heat-shock and general stress response in *Bacillus subtilis*. *Mol. Microbiol.*, 19: 417-428.
16. Bernhardt, J., U. Volker, A. Volker, H. Antelmann, R. Schmid, H. Mach and M. Hecker, 1997. Specific and general stress proteins in *Bacillus subtilis* a two-dimensional protein electrophoresis study. *Microbiol.*, 143: 999-1017.
17. Gurr, M.I. and J.L. Harwood, 1991. Chapter 8 Lipid Functions from Lipid Biochemistry, An introduction, 4th Edn. Chapman and Hall, London, New York, Tokyo.
18. Tatzer, V., G. Zellnig, S.D. Kohlwein and R. Schneiter, 2002. Lipid-dependent subcellular relocalization of the acyl chain desaturase in yeast. *Mol. Biol. Cell*, 13: 4429-4442.
19. Lee, K., 2004. Cold shock response in *Lactococcus lactis* ssp. *Diacetylactis*: A comparison of the protection generated by brief pre-treatment at less severe temperatures. *Process Biochem.*, 39: 2233-2239.
20. Aguilar, P.S., P. Lopez and D.de Mendoza, 1999. Transcriptional control of the low-temperature-inducible *des* gene, encoding the $\Delta 5$ desaturase of *Bacillus subtilis*. *J. Bacteriol.*, 181: 7028-7033.

Conservation Priority of the Threatened Plants in the Lower Tersakan Valley (A5 Amasya - Turkey) and Its Floristic Diversity

Ferhat Celep and Musa Dogan

Department of Biological Sciences, Middle East Technical University, 06531, Ankara, Turkey

Abstract: The natural flora of lower Tersakan Valley, located in Amasya province (A5) in northern Turkey, was studied between 2001 and 2004. During the floristic surveys, 457 taxa of 301 genera belonging to 74 families were recorded and 54 of these determined to be threatened according to IUCN Red List Categories. The types of the threat categories of these taxa are as follows: 1 taxon Critically Endangered, 3 taxa, Endangered, 1 taxon Vulnerable, 5 taxa Near Threatened and 44 taxa Least Concern. A total of 50 of the taxa (10.94%) determined in the research area are endemic. The distribution of taxa according to phytogeographic regions is as follows: Irano-Turanian elements 77 (16.8%), Euro-Siberian elements 39 (8.5%), Mediterranean elements 35 (7.6%), widespread and unknown 306 (66.9%). Four vegetation types can be recognized in the study area: *Degraded forest, maquis, steppe* and *riparian* vegetation. Unfortunately, this rich plant diversity faces various threats such as habitat destruction, urbanization, environmental contamination and cultivation. Urgent actions are needed for conservation of the lower Tersakan Valley's plant biodiversity and vegetation types.

Key words: Conservation . lower Tersakan valley . Threatened habitats . Turkey

INTRODUCTION

Turkey has rather interesting flora. In the country, nearly one in every three plants in Turkey is endemic, an astonishingly high percentage for a mainland country. The exceptional diversity in Turkey's flora is the collective results of extent of a variety of climates, topographical diversity with marked changes in ecological factors over a short distance, geological and geomorphic variation, a range of aquatic environments such as seas, lakes and rivers, altitude variations from sea level to 5000 m. There are a number of major mountain ranges in Anatolia that constitute effective barriers and these have further encouraged a greater diversity of species particularly in the inner ecosystems due to isolation [1, 2].

Amasya (A5 *sensu* [3]) is a small city and located in the northern Turkey. The region has an area of 5690 km² and altitude ranges between 370 and 2135 m. Amasya consists of a number of mountains, plains, lakes, rivers, streams, valleys and plateaus. For these reasons, the area shows very interesting geographic, edaphic, geologic and climatic diversity.

In addition, *meadows, wetlands, forests, maquis, steppes, riparian* and different vegetation types appearing in vertical belts are also important. An estimated over 1000 species of vascular plants are

found in Amasya, almost 200 of which are endemic [3, 4]. These figures make this region one of the richest and interesting botanical area among the territories of Northern Turkey.

Main research area, lower Tersakan Valley, is situated in the northern part of the city center and it appears to be a transitional zone between Central Anatolia steppes and the North Anatolia. Moreover, it is a transitional zone between Euro-Siberian and Irano-Turanian phytogeographic regions. Such transitional zones have interesting properties, due to the mixing of oceanic and continental climates. This situation is clearly reflected in the flora and vegetation of the study area. In certain places of the region Mediterranean climate is seen and Mediterranean enclaves are widespread in the study area [5].

This research has a great importance for a better understanding of human impact on the floristic changes caused by the land use policies in the region and certainly it has some advantages for sustainability. In addition, the factors affecting survival of the threatened plant species are explained along with the sustainable use of plant diversity, some conservation priorities and strategies are also suggested.

Study area: Main research area, lower Tersakan Valley, is located in the northern part of Amasya

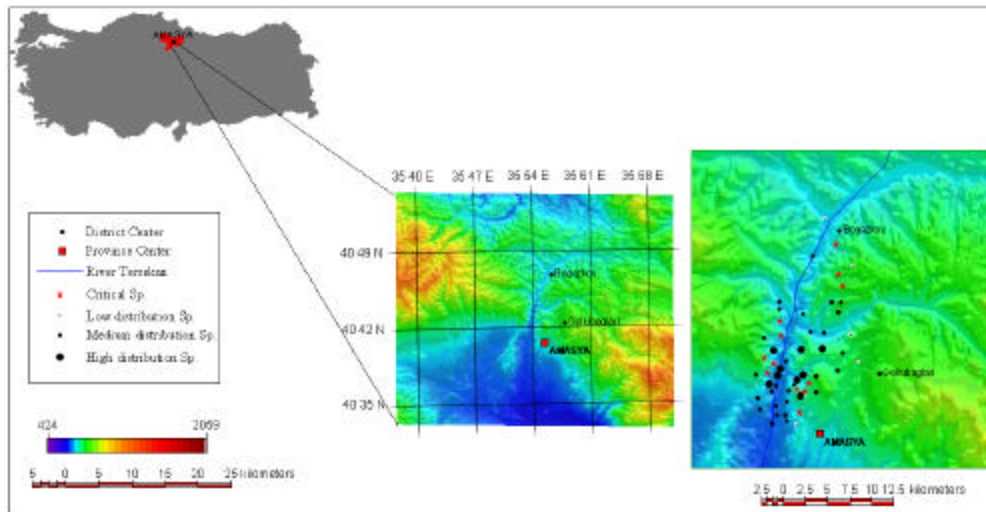


Fig. 1: Location of the study area, the distribution of 54 threatened plant populations according to their rarity

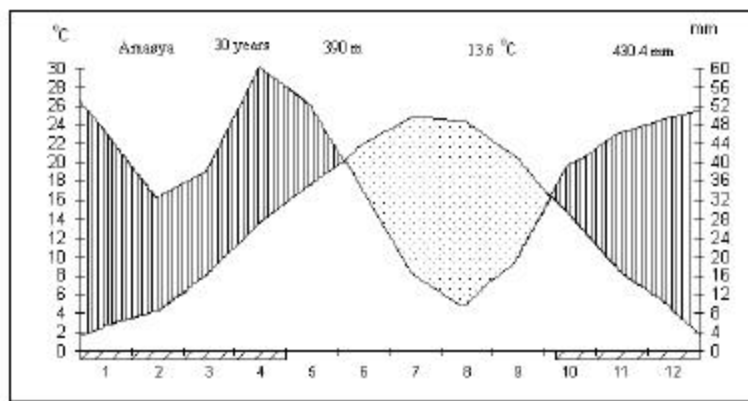


Fig. 2: Ombrothermic diagram of Amasya

province and lies approximately at 40°39' 24"-40°43' 55" north latitudes and 35°50' 03"-35°46' 24" east longitudes. Altitude of the study area varies between 390 m and 1130 m and it appears to be generally mountainous. Tersakan river is placed in the centre of the valley and flows from north to south direction, towards Yesilirmak river. In addition, the study area lies on the ancient silk road (Fig. 1).

The climate of the research area is based on the data obtained from the meteorology station in Amasya. The dominant bioclimate is characterized as a semi-arid Mediterranean climate. The Mediterranean climate is experienced by hot and dry summers followed by cold and wet winters [6]. Rainfall is lower from the north to the south of the valley [7]. The mean annual average temperature is 13.6°C and precipitation is 430.4 mm. It can be seen that heavy rainfall is received in November to April, while the dry period extends from the beginning of June until the end of October. The most of

precipitation occurs in the Spring and Winter. The ombrothermic diagram shows the months with dry and rainy period (Fig. 2).

The geological structure of the research area mostly consists of calcareous rock which precipitate, on the paleozoic old basic rocks [8]. There are five large soil groups in the study area: brown forest soil, chesnut colour, brown soil, alluvial and colluvial soil [9].

MATERIALS AND METHODS

Field observations were conducted and plant species were gathered to depict the flora of the area and make an inventory of plants before habitat degradation in 2001 and 2004. Efforts were made to collect both flowering and fruiting specimens. The specimens were prepared according to established herbarium techniques. The Flora of Turkey [3, 10, 11] and other related floras [12, 13] were utilized in the

identification of the specimens. Experts were consulted in some controversial cases. The authorities are cited using Authors of Plant Names [14].

Threatened categories are proposed for endemic and rare taxa in the study area according to IUCN risk categories [15, 16]. The distribution of threatened plant populations was also mapped (Fig. 1). The following abbreviations are used; EN, Endangered; VU, Vulnerable; CR, Critically endangered; LC, Least concern; NT, Near threatened.

RESULT AND DISCUSSION

During the floristic study, about 1000 vascular plant specimens were collected from lower Tersakan Valley and its vicinity, 325 species (457 taxa) in 301 genera, belonging to 74 families were established. Four taxa belong to Gymnospermae, while the other 453 were in Angiospermae [4].

A total of 54 threatened plant species, belonging to 43 genera in 17 families, were found. The distribution of the threat categories of these taxa is as follows: 1 taxon CR, 3 taxa EN, 1 taxon VU, 5 taxa NT and 44 taxa LC (Fig. 3). The largest families are Asteraceae, Fabaceae, Lamiaceae, Boraginaceae, Brassicaceae and Scrophulariaceae, together comprising about 60% of

the total threatened species (Fig. 4). More than 35% of the threatened species belong to 8 genera, including *Alyssum*, *Minuartia*, *Astragalus*, *Onobrychis*, *Centaurea*, *Scorzonera*, *Onosma* and *Asperula*.

Of the total threatened species in the valley, about 77.8% is distributed between 400-650 m, 14.8% between 650-850 m and 7.4% above 850 m, reflecting that most of the threatened species in the valley could not survive in a wider vertical range with stronger adaptation. Urbanization and cultivation activities have been concentrated at the lower part of the valley; therefore, human activities are directly effect on the threatened plant species and urgent conservation measures are needed for the sustainability of threatened species.

Of the total threatened species, 64.8% grows in *steppe*, 16.6% grows in *maquis* and the remaining 1.8% grows in *riparian* habitats. 16.7% of them were found in more than two kinds of habitats. This shows that the *steppe* is the main habitat for threatened plants and therefore protection of the *steppe* seems to be the most important vegetation for saving these species. The threatened flora of the study area with threatened species, their conservation status, vegetation types and elevation ranges are given in Table 1.

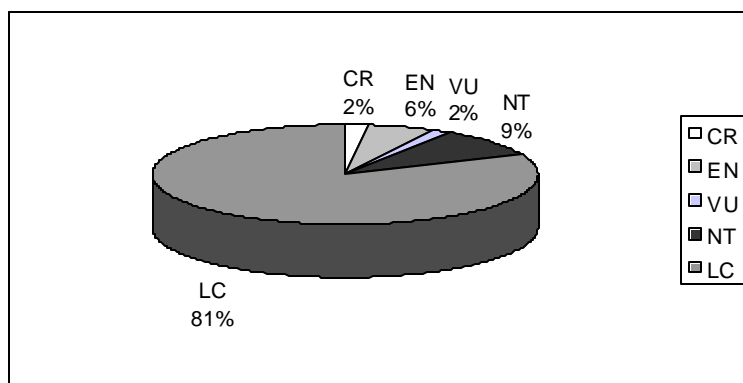


Fig. 3: Distribution of the threatened species according to IUCN Red List categories

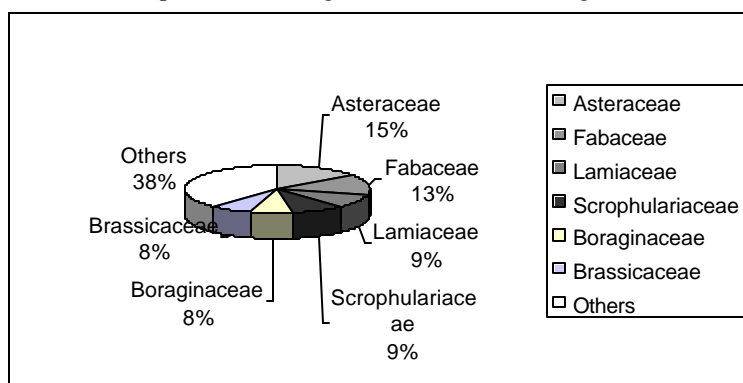


Fig. 4: The richest families according to threatened species

Table 1: The threatened flora of the study area, its IUCN red data list categories, vegetation types and elevational range

Species name	Conservation status	Vegetation types	Elevational range (m)
Endemic species			
<i>Delphinium venulosum</i> Boiss.	LC	Steppe	400-650
<i>Consolida thirkeana</i> (Boiss.) Schröd	LC	Steppe	400-650
<i>Alyssum blepharocarpum</i> Dudley & Hub.-Mor.	NT	Steppe	400-650
<i>Alyssum praecox</i> Boiss. et Bal. var. <i>praecox</i> Boiss.	LC	Steppe	400-650
<i>Draba rigida</i> Willd. var. <i>rigida</i>	LC	Maquis (on rocks)	400-650
<i>Erysimum amasianum</i> Hausskn. et Bornm.	EN (B1 a,b and B2 a,b)	Steppe	400-650
<i>Minuartia erythrosepala</i> (Boiss.) Hand.-Mazz. var. <i>cappadocica</i> (Boiss.) McNeill	LC	Steppe (on rocks)	400-650
<i>Minuartia corymbulosa</i> (Boiss & Bal.) McNeil. var. <i>Corymbulosa</i>	NT	Steppe (on rocks)	400-650
<i>Saponaria prostrata</i> Willd. subsp. <i>Prostrata</i>	LC	Steppe	400-650
<i>Haplophyllum armenum</i> Spach	LC	Steppe	400-650
<i>Astragalus dipsaceus</i> Bunge	LC	Steppe	400-650
<i>Astragalus leucothrix</i> Freyn & Bornm.	LC	Steppe	400-650
<i>Astragalus lycius</i> Boiss.	NT	Steppe	400-650
<i>Astragalus squalidus</i> Boiss. & Noe	LC	Steppe	650-850
<i>Hedysarum pogonocarpum</i> Boiss.	LC	Steppe	400-650
<i>Onobrychis bornmuelleri</i> Freyn	EN (B1 a,b and B2 a,b)	Steppe	400-650
<i>Onobrychis cappadocica</i> Boiss.	LC	Steppe	650-850
<i>Heracleum platytaenium</i> Boiss.	LC	Maquis (on rocks)	+850
<i>Inula anatolica</i> Boiss.	LC	Steppe (on rocks)	400-650
<i>Anthemis sintenisii</i> Freyn	LC	Steppe	400-650
<i>Tripleurospermum rosellum</i> (Boiss & Orph.) Hayek var. <i>album</i> E. Hossain	VU (B1 a,b and B2 a,b)	Steppe	400-650
<i>Jurinea pontica</i> Hausskn. et Freyn ex Hausskn.	LC	Steppe	650-850
<i>Centaurea consanguinea</i> DC.	LC	Steppe	+850
<i>Scorzonera acuminata</i> Boiss.	LC	Steppe, Degraded forest	400-650
<i>Scorzonera amasiana</i> Hausskn. & Bornm.	CR (B1 a,b and B2 a,b)	Steppe (on rocks)	50-850
<i>Campanula saxonorum</i> Gandoger	LC	Steppe	400-650
<i>Asyneuma limonifolium</i> (L.) Janchen subsp. <i>Pestalozzae</i> (Boiss.) Damboldt	LC	Maquis, Degraded forest	400-650
<i>Vincetoxicum fuscatum</i> (Hornem.) Reichb. Fil. subsp. <i>Boissieri</i> (Kun.) Browicz	LC	Maquis, Steppe	400-650
<i>Convolvulus assyricus</i> Griseb.	LC	Maquis, Steppe	400-650
<i>Paracaryum ancyritanum</i> Boiss.	LC	Steppe	400-650
<i>Echium orientale</i> L.	LC	Steppe	400-650
<i>Onosma bornmuelleri</i> Hausskn.	LC	Steppe	400-650
<i>Onosma stenobum</i> Hausskn. ex H. Riedl	LC	Steppe	400-650
<i>Verbascum myrianthum</i> Boiss.	EN (B1 a,b and B2 a,b)	Maquis	650-850
<i>Scrophularia libanotica</i> Boiss. subsp. <i>libanotica</i> var. <i>pontica</i> R. Mill	LC	Riparian	400-650
<i>Linaria corifolia</i> Desf.	LC	Steppe	400-650
<i>Digitalis lamarekii</i> Ivan.	LC	Steppe, Degraded forest	+850
<i>Scutellaria salviifolia</i> Bentham	LC	Steppe	400-650
<i>Phlomis armeniaca</i> Willd.	LC	Steppe, Maquis	400-650
<i>Lamium ponticum</i> Boiss. et Bal. ex Boiss.	LC	Steppe	400-650
<i>Sideritis amasiaca</i> Bornm.	NT	Maquis	650-850
<i>Salvia cyanescens</i> Boiss. & Ball.	LC	Steppe	400-650
<i>Asperula pestalozzae</i> Boiss.	LC	Steppe (on rocks)	400-650
<i>Asperula suavis</i> Fisch. et Mey.	LC	Maquis	650-850
<i>Galium fissurense</i> Ehrend. et Schönb.-Tem.	LC	Maquis	400-650
<i>Arum euxinum</i> R. Mill	LC	Maquis, Degraded forest	+850
<i>Allium cappadocicum</i> Boiss	LC	Maquis	400-650
<i>Bellevalia gracilis</i> Feinbrun	LC	Steppe	400-650
<i>Hyacinthella micrantha</i> (Boiss.) Chouard	NT	Maquis	400-650
<i>Iris galatica</i> Siehe	LC	Steppe, Maquis	400-650
<i>Crocus ancyrensis</i> (Herbert) Maw	LC	Maquis, Steppe	400-650
Non Endemic-Rare species <i>Centaurea urvillei</i> DC. subsp. <i>steppeposa</i> Wagenitz	LC	Maquis	400-650
<i>Astragalus densifolius</i> Lam. subsp. <i>amasiensis</i> (Freyn) Aytac	LC	Steppe	650-850
<i>Veronica multifida</i> L.	LC	Steppe	400-650

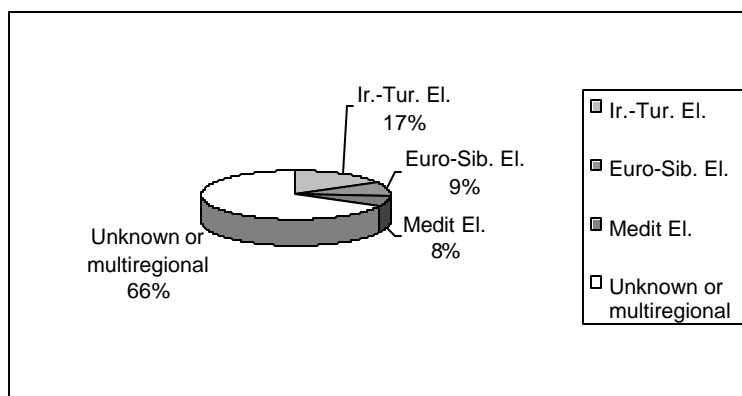


Fig. 5: Phytogeographic distribution of the taxa in the study area

According to the latest IUCN risk categories and the field observations, *Scorzonera amasiana* Hausskn. & Bornm. is Critically endangered [CR (B1 a,b and B2 a,b): extent of occurrence less than 100 km²; area of occupancy less than 10 km²; known to exist at only a single location; inferred decline in the area, extent and/or quality of habitat]. *Verbascum myrianthum* Boiss., *Onobrychis bornmuelleri* Freyn and *Erysimum amasianum* Hausskn. et Bornm. are Endangered [EN (B1 a,b and B2 a,b): extent of occurrence less than 5000 km², area of occupancy less than 500 km², known at no more than five locations; inferred decline in the area, extent and/or quality of habitat]. *Tripleurospermum rosellum* (Boiss & Orph.) Hayek var. *album* E. Hossain is Vulnerable [VU (B1 a,b and B2 a,b): Extent of occurrence less than 20,000 km²; area of occupancy less than 2000 km², known at no more than 10 locations; inferred decline in the area, extent and/or quality of habitat]. *Hyacinthella micrantha* (Boiss.) Chouard, *Sideritis amasiaca* Bornm., *Astragalus lycius* Boiss., *Minuartia corymbulosa* (Boiss & Bal.) McNeil var. *corymbulosa* and *Alyssum blepharocarpum* Dudley & Hub.-Mor. are Near Threatened (NT) and remaining Least Concern (LC) [16].

The vegetation of the lower Tersakan valley is rather variable. Four main vegetation types can be distinguished in the study area as a result of this research: *Degraded forest*, *maquis*, *steppe* and *riparian* vegetation. Vegetation is stratified from the bottom of the valley to the slopes. Altitude, direction, topography, temperature and precipitation play an important role in stratification.

Degraded forest vegetation is especially widespread in the northern-exposed slopes of the area and includes *Quercus pubescens* Willd., *Carpinus orientalis* Miller and *Juniperus oxycedrus* L forests formed as a result of the destruction of *Pinus brutia* Ten forest. *Maquis vegetation* is found in the area as

Mediterranean enclaves because of the destruction of *Pinus brutia* ten forest. *Maquis vegetation* usually occurs on south-facing slopes of the area and lower part of the valley. *Steppe vegetation*, most widespread, embodies perennial herbaceous and semi-woody dwarf plants dominating the south facing slopes, under severe urbanization and cultivation pressure. *Riparian vegetation*, only in the Tersakan River bank, including herbaceous or woody plants such as *Populus alba* L., *Salix triandra* L. subsp. *bornmuelleri* (Hausskn.) A. Skv. The characteristic species of vegetation types, their phytogeographic regions and elevation range are given in Table 2.

The distribution of taxa according to phytogeographical regions is as follows: Irano-Turanian elements 77 (16.8%), Euro-Siberian elements 39 (8.5%), Mediterranean elements 35 (7.6%), unknown or multiregional 306 (66.9%) (Fig. 5). Irano-Turanian and Mediterranean elements were generally distributed in open and steppe areas, whereas Euro-Siberian elements were found in humid shadowy areas, around damp springs and in meadows.

The first five families with the highest number of taxa are Asteraceae (56 spp.) (12.2%), Fabaceae (42 spp.) (9.2%), Lamiaceae (35 spp.) (7.6%), Brassicaceae (33 spp.) (7.2%) and Poaceae (33 spp.) (7.2%). Five of the richest genera are *Astragalus* L. (7 spp.), *Alyssum* L. (7 spp.), *Vicia* L. (6 spp.), *Salvia* L. (6 spp.) and *Centaurea* L. (5 spp.).

Natural ecosystems degrade and decline rapidly as human populations increase. Due to the rapid population increase in Turkey within the last few decades many natural habitats have been fragmented, reduced in size, degraded or destroyed [17]. Similarly, the destruction of habitat through human encroachment is the principal cause of the loss of the area's biodiversity [18]. Habitat loss, clearing of the natural vegetation for urbanization, cultivation and pollution are the main causes of threats in the study area.

Table 2: The characteristic species of vegetation types, their phytogeographic regions and elevational ranges

Main vegetation types	Elevational range (m)	Characteristic species	Phytogeographic element		
Degraded forest	450-900	<i>Pinus brutia</i> Ten	Unknown or multiregional		
		<i>Quercus pubescens</i> Willd.	Unknown or multiregional		
		<i>Quercus robur</i> L.	Euro-Siberian El.		
		<i>Carpinus orientalis</i> Miller	Unknown or multiregional		
		<i>Juniperus oxycedrus</i> L.	Unknown or multiregional		
		Maquis	450-650 (800)	<i>Phillyrea latifolia</i> L.	Mediterranean El.
				<i>Cistus creticus</i> L.	Mediterranean El.
				<i>Jasminum fruticans</i> L.	Mediterranean El.
				<i>Pistacia terebinthus</i> L.	Mediterranean El.
				<i>Quercus cerris</i> L.	Mediterranean El.
<i>Rhamnus oleoides</i> L.	Mediterranean El.				
<i>Paliurus spina-christii</i> Miller	Unknown or multiregional				
<i>Colutea cilicica</i> Boiss & Ball.	Unknown or multiregional				
Steppe	450-900			<i>Astragalus microcephalus</i> Willd.	Irano-Turanian El.
				<i>Acantholimon acerosum</i> (Willd.) Boiss.	Irano-Turanian El.
		<i>Stipa ehrenbergiana</i> Trin. & Rupr.	Irano-Turanian El.		
		<i>Thymus sipyleus</i> Boiss.	Unknown or multiregional		
		<i>Centaurea solstitialis</i> L.	Unknown or multiregional		
		<i>Xeranthemum annuum</i> L.	Unknown or multiregional		
		<i>Chardinia orientalis</i> (L.) O. Kuntze	Irano-Turanian El.		
		<i>Onosma sericeum</i> Willd.	Irano-Turanian El.		
		<i>Teucrium polium</i> L.	Unknown or multiregional		
		<i>Teucrium chamaedrys</i> L.	Irano-Turanian El.		
		<i>Verbascum orientale</i> (L.) All.	Mediterranean El.		
		<i>Globularia trichosantha</i> Fish. & Mey.	Unknown or multiregional		
		<i>Pennisetum orientale</i> L. C. M. Richard	Irano-Turanian El.		
		<i>Chrysopogon gryllus</i> (L.) Tri.	Unknown or multiregional		
		<i>Convolvulus assyriacus</i> Griseb.	Irano-Turanian El.		
(on rocks)	400-700	<i>Onosma alba-roseum</i> Fisch. & Mey.	Unknown or multiregional		
		<i>Sedum album</i> L.	Unknown or multiregional		
		<i>Sedum hispanicum</i> L.	Unknown or multiregional		
		<i>Paronychia kurdica</i> Boiss.	Unknown or multiregional		
		<i>Minuartia erythrosepala</i> Hand.-Mazz.	Irano-Turanian El.		
		Riparian	390-450	<i>Elaeagnus angustifolia</i> L.	Unknown or multiregional
<i>Salix triandra</i> L. subsp. <i>bornmuelleri</i> A.Skv.	Irano-Turanian El.				
<i>Mentha longifolia</i> (L.) Hudson	Euro-Siberian El.				
<i>Plantago lanceolata</i> L.	Unknown or multiregional				
<i>Lythrum salicaria</i> L.	Euro-Siberian El.				
<i>Epilobium montanum</i> L.	Euro-Siberian El.				
<i>Polygonium lapathifolium</i> L.	Unknown or multiregional				
<i>Reseda luteola</i> L.	Unknown or multiregional				
<i>Sambucus ebulus</i> L.	Euro-Siberian El.				
<i>Conium maculatum</i> L.	Unknown or multiregional				
<i>Schonoplectus lacustris</i> (L.) Palla	Unknown or multiregional				
<i>Dipsacus laciniatus</i> L.	Unknown or multiregional				
<i>Phragmites australis</i> (Cav.) Trin. ex Steudel.	Unknown or multiregional				
		<i>Cyperus longus</i> L.	Unknown or multiregional		

Because of the rapidly growing human population in the city center and the nearby lowlands, recently, the area has been declared as a residential area by the municipality. Afterwards, construction activities have been gradually increased. Nowadays, majority of the valley basin is occupied by construction companies, especially; lower part of the valley is almost degraded. Consequently, in near future, plant

diversity will decline and threatened species will disappear in the area.

Another factor affects biodiversity in the area is the presence of factories which are situated at the upper part of the Tersakan River. From time to time, these factories release some toxic waste into the river. This detrimental situation gives rise to the decline of both floral and faunal diversity in the area.

CONCLUSIONS

As a result of this study, it can be expected that gradually the important floristic changes will take place in the area due to the habitat destruction. Urbanization is one of the leading causes of species extinction. Many human activities promote biotic homogenization, but urbanization is one of the most homogenizing activities of all [19, 20]. Consequently, in near future, plant diversity will decline and threatened species will disappear in the area if necessary conservation measures are not taken. Although in recent years local government authorities have made some efforts to preserve biodiversity, but much work remains to be done. The area needs to be legally protected with protection of the small population and vegetation, besides the area is urgently modeled and managed by means of using the Geographical Information System (GIS) images. In addition, several other measures need to be considered such as a search for the threatened species in surrounding areas, rehabilitation or restoration of damaged habitats, transferring the species in surrounding protected areas and cultivation in botanical gardens.

ACKNOWLEDGEMENTS

We thank to Prof. Dr. Zeki Kaya, Prof. Dr. H. Resit Akçakaya and Prof. Liangdong Guo, for providing useful discussions and their constructive critics about the manuscript, to Research Assistant Banu Kaya for drawing map. This project supported by the Gazi University Scientific Research Project Unit.

REFERENCES

1. Davis, P.H. and I.C. Hedge, 1971. Distribution patterns in Anatolia with particular reference to endemism. *Plant life of South-West Asia*, pp: 15-27.
2. Davis, P.H. and I.C. Hedge, 1975. *Flora of Turkey: Past, present and future*. *Candollea*, 30: 331-351.
3. Davis, P.H. *et al.*, 1965-1985. *Flora of Turkey and the East Aegean, Islands 1-9*. Edinburgh University Press, Edinburgh, UK.
4. Celep, F., Z. Aytac and F. Karaer, 2006. Plant Diversity and Distribution in the Lower Tersakan Valley. *Flora Mediterranean*, 16: 295-332
5. Karaer, F. and M. Kiling, 2001. The Flora of Kelkit Valley, *Tr. J. Bot.*, 25: 195-238.
6. Akman, Y., 1990. *Iklim ve Biyoiklim*. Palme Yayinlari Da, No. 103, Ankara, Turkey.
7. Anonymus, 1984. *Ortalama Ekstrem Sicaklik ve Yagis Degerleri Bülteni*, Meteoroloji Isleri Genel Müdürlüğü Yayini, Ankara, Turkey.
8. Eser, S., 1983. *Merzifon Depresyonu ve Çevresinin Jeomorfolojik Etüdü*, İstanbul Üniversitesi Edebiyat Fakültesi, Yayin No: 3100, İstanbul.
9. KHGM, 2002. *National Digital Soil Data Base of Turkey*. The Ministry of agriculture and Rural affair of Turkey. The General Directorate of Rural Services, Ankara.
10. Davis, P.H., R. Mill and K. Tan, 1988. *Flora of Turkey and the East Aegean Islands 10 (Suppl.)*. Edinburgh University Press, Edinburgh, UK.
11. Güner, A., N. Özhatay, T. Ekim and K.H.C. Baser, 2000. *Flora of Turkey and the East Aegean Islands*. Edinburgh University Press, 11(Suppl.), Edinburgh, UK.
12. Townsend, C.C. and G. Evan, 1968-1980. *Flora of Iraq, Baghdad, Iraq*, pp: 1-9.
13. Tutin, G.T. *et al.*, 1964-1980. *Flora Europaea vols. I-V*, Univ. Press, Cambridge.
14. Brummit, R.K. and C.E. Powel, 1992. *Authors of Plant Names*. Royal Botanic. Gardens, Kew, London.
15. Ekim, T., M. Koyuncu, M. Vural, H. Duman, Z. Aytac and N. Adigüzel, 2000. *Türkiye Bitkileri Kırmızı Kitabı (Egrelti ve Tohumlu Bitkiler)*, Red Data Book of Turkish Plants (Pteridophyta and Spermatophyta). Turkish Association for the Conservation of Nature-Van Centennial University, Ankara, Turkey.
16. IUCN, 2001. *IUCN Red List Categories and Criteria: Version 3.1*. Prepared by the IUCN Species Survival Commission. IUCN, Gland, Switzerland, li + 30 pp.
17. Kaya, Z. and D.J. Raynal, 2001. Biodiversity and conservation of Turkish forests. *Biological Conservation*, 97: 131-141.
18. López-Pujol, J., F.M. Zhang and S. Ge, 2006. Plant biodiversity in China: Richly varied, endangered and in need of conservation. *Biodiversity and Conservation* DOI 10.1007/s10531-005-3015-2.
19. Czech, B., P.R. Krausman and P.K. Devers, 2000. Economic associations among causes of species endangerment in the United States. *BioSci.*, 50: 593-601.
20. McKinney, M.L., 2006. Urbanization as a major cause of biotic homogenization. *Biological Conservation*, 127: 247-260.

Potential Effects of Prolonged Ultraviolet Radiation Exposure in Plants: Chloroplast DNA Analysis

¹S.W. Mpoloka, ²V.A. Abratt, ²S.G. Mundree, ²J.A. Thomson and ³C.F. Musil

¹Department of Biological Sciences, University of Botswana, Private Bag UB 00704 Gaborone, Botswana

²Department of Molecular and Cell Biology, University of Cape Town, P/Bag Rondebosch 7700, RSA

³Stress Ecology Research Unit, National Botanical Institute, P/Bag X7 Claremont 7735, Cape Town, RSA

Abstract: The present study on the Namaqualand daisy, *Dimorphotheca sinuata* sought to address two main questions: first whether the natural populations show any evidence of variation in the chloroplast genome and secondly if the changes could be attributed to prior damage by UV-B *i.e.* via the formation of pyrimidine dimers at some stage in their history. Characterization of chloroplast DNA from natural plant populations of *D. sinuata* across a latitudinal gradient was carried out using restriction endonuclease digestion. The enzymes used included *Dra*I (TTTAAA), *Eco*RI (GAATTC) and *Hind*III (AAGCTT) whose recognition sequences are possible targets for UV-B radiation and *Bam*HI (GGATCC) and *Eco*RV (GATATC), whose recognition sequences are not obvious UV-B targets. Plants growing at northern latitudes (potentially higher UV-B environments) revealed striking polymorphisms that may be attributed to genome re-arrangements resulting from UV-B stress when compared with plants from southern latitudes (lower UV-B environments). This is the first known attempt at developing a Southern African biological method for predicting the long-term effects of ozone depletion and the resultant rise in UV-B radiation, on our indigenous flora.

Key words: Chloroplast DNA . UV-B . DNA damage . stress

INTRODUCTION

Significant latitudinal variation in incident UV-B radiation has been reported [1, 2]. However, a few studies have been carried out in which natural plant performance across natural solar UV-B gradients at different elevations was compared. Variable sensitivities to UV-B radiation have been reported which implies the presence of natural adaptations to UV-B stress [3, 4]. These studies indicate that species and ecotypes from high UV-B irradiance environments are often less sensitive to elevated UV-B radiation than those from low UV-B irradiance locations [2, 4]. This has led to suggestions that genotypic differentiation may have developed among plants along these gradients. Previous studies of plants grown for several generations in the presence of enhanced UV-B radiation showed evidence of UV-B effects on various physiological processes, growth and reproduction, indicating a likelihood of these effects being heritable [5-7]. UV-B radiation has been reported to cause several lesions in DNA including double strand breaks whose induction in turn increases the frequency of homologous recombination, hence genome rearrangements [8, 9]. Studying UV

adaptations in natural plant populations could enable us to find novel or unique protective mechanisms that have not been detected in crop plants already exposed to intensive artificial selection. Perhaps the first place to search for UV-B responsiveness in native plants is in regions where natural levels of UV-B are already quite high. Plants that naturally occur in high UV environments would undoubtedly have evolved specific adaptations that protect them from the deleterious effects of UV-B radiation [3]. However, this does not mean that they do not respond to UV-B: Indeed it might suggest quite the opposite in that their anti-UV mechanisms may be permanently induced. Such plants could also show reduced responsiveness mainly because of reduced sensitivity to UV-B radiation and possibly by possessing some adaptive mechanisms against UV-B radiation.

UV-B induced reductions in pollen viability in several South African annual species grown under enhanced UV-B have been reported [10] and it has been suggested that, even under experimental treatments using natural light, damage to the plant genome caused by elevated UV-B may also be inherited by successive generations of the desert annual *D. sinuata* and thus

accumulate in the genetic material [9]. This form of damage may be extremely important in populations which have rapid turnover of generations such as annual species which are common in high-radiation desert environments. Furthermore, populations which are isolated by habitat fragmentation may be further at risk to this form of damage due to limited out crossing opportunities.

Based on observations that plant exposure to episodic or steadily increasing doses of UV-B damages photosynthetic reaction centres, cross-links cellular proteins and induces mutagenic DNA lesions, it was proposed that *D. sinuata* plants that occur naturally at higher latitudes associated with higher UV-B levels may be physiologically and reproductively less sensitive to UV-B radiation. Plants are unique in their ability to obtain energy directly from sunlight for photosynthesis and, as a result, are subject to continuous exposure to the ultraviolet (UV) radiation that is present in the spectrum of solar radiation. Unlike animals, plants do not sequester a germ line early in development. Thus a stress-induced mutation in any cell that later gives rise to reproductive tissue can be passed onto the next generation. Such heritable stress-induced somatic mutations may play a potential role in the evolutionary process. Genetic changes induced by environmental stress and the potential impact these

changes have on organismal evolution are areas of both great interest and controversy. Stress-induced mutations have been documented in many organisms [11]. Unfortunately, the mechanisms that generate these mutations, the type of stress-induced mutations that occur in plants and whether or not these mutations are inherited and thus of evolutionary significance is still unknown.

By virtue of being the light energy harvesting machinery of the plant, chloroplasts have a relatively greater potential for acquiring ultraviolet induced genetic damage than other organelles. It was therefore decided that chloroplast DNA (ctDNA) from natural populations would be analysed. The present study describes investigations into the genetics of long term UV-B exposure in Namaqualand daisies from Southern Africa and the aim was to establish if indigenous plants are endangered by prolonged ultra-violet radiation exposure.

MATERIALS AND METHODS

Seeds used to generate study plants were collected from three different sites in the Republic of South Africa. The northern-most site was Augrabies Falls (28°38'S, 20°25'E) and the southern-most was Kirstenbosch Botanical Gardens, Cape Town (35°12'S,



Fig. 1: Location of sites from which samples were collected

18°25'E). Seeds for the Kirstenbosch population were collected from the wild several generations in the past and were propagated in the Kirstenbosch Botanical Gardens. Samples were also collected from Bitterfontein, representing the mid-latitudes (Fig. 1).

Seeds were soaked for five minutes in a 5% solution of sodium hypochlorite and rinsed five times in distilled water. The seeds were then placed on five layers of moistened Whatman filter paper on Petri dishes and these were sealed with paraffin-wax film to minimize evaporation. Seeds were germinated in the dark for three days before being potted in potting medium comprising coarse sand, leaf mould and loam (2:1:1, v/v) and were watered with tap water daily thereafter. The standard conditions in the growth room were as follows: temperature = 22°C, relative humidity = 65%, 16 hours light and 8 hours darkness with an intensity of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. After six weeks, leaf samples were taken for chloroplast DNA isolation.

Chloroplast DNA was isolated from fresh plant material by first enriching for the chloroplast fraction in sucrose gradients [12]. 5 μg aliquots of ctDNA were cut to completion with the following restriction enzymes (RE): *Bam* HI, *Dra* I, *Eco* RI, *Eco* RV and *Hind* III (Roche Molecular Diagnostics). The entire digest was then loaded onto a 25 cm-long, 0.8% agarose gel and subjected to electrophoresis for 16 hours at 30V to estimate the ctDNA genome size and to compare the

RE digestion patterns of plants from different latitudes. Samples were divided into two and were separated by electrophoresis on 0.6% and 1.5% agarose gels to resolve the high molecular weight and low molecular weight fragments respectively. Alternatively, digests were resolved by electrophoresis on 0.8% agarose gels, blotted onto a positively charged nylon membrane and probed with a DIG-labelled *Bam* HI digest of ctDNA.

RESULTS AND DISCUSSION

The chloroplast DNA digest is shown in Fig. 2. The gel was probed with DIG-labelled chloroplast DNA that had been digested with *Bam* HI. Lanes 1 and 2 = *Dra* I, lanes 3 and 4 = *Bam* HI, lanes 5 and 6 = *Eco*RV. The chloroplast genome size of *D. sinuata* was estimated to be 123.80 \pm 11.57 kb and lies within the range reported for chloroplast DNA sizes of higher plants (80-200 kb) [13].

Chloroplast DNA restriction endonuclease analysis was used in this study because T=T sites in the DNA were being proposed as potential candidates for indicating UV-B effects. This is because the chloroplast genome, by virtue of being housed in the light harvesting apparatus, is likely to be targeted by the damaging effects of UV-B radiation. Chloroplast DNA also has a relatively higher likelihood of acquiring UV-induced genetic damage, especially at T=T sites. This

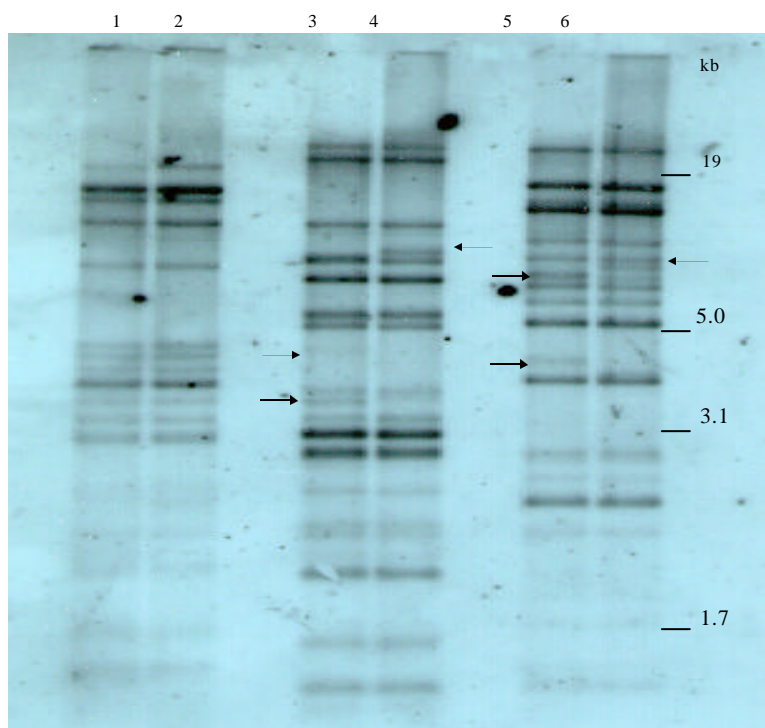


Fig. 2: Southern blot of chloroplast DNA. Samples from Kirstenbosch (lanes 1, 3 and 5) and Augrabies Falls (lanes 2, 4 and 6). The arrowheads indicate the polymorphic bands from the different restriction endonucleases. Fragment sizes are indicated on the right of the figure in kilo bases

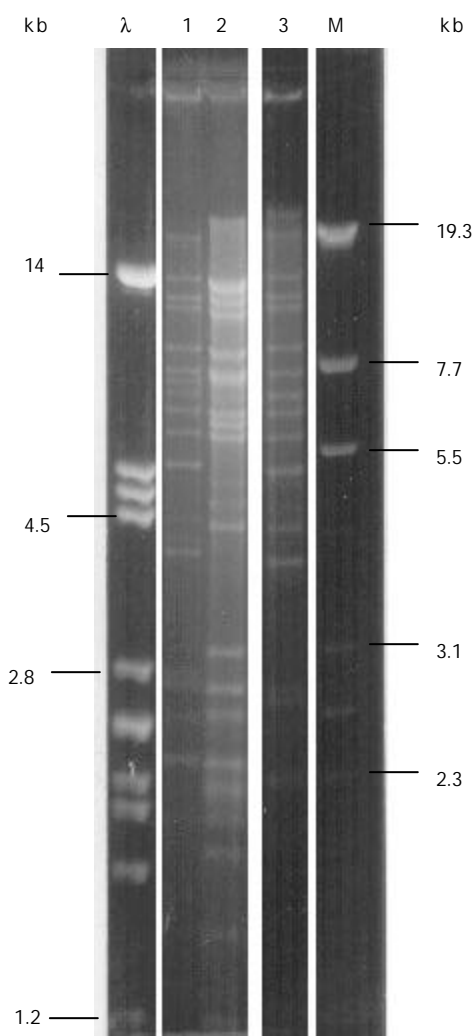


Fig. 3: Chloroplast DNA restriction endonuclease analysis

could be tested using restriction endonucleases which target T=T sites in the DNA such as *Dra*I, *Eco*RI and *Hind*III. However, DNA samples showed polymorphisms with enzymes that do not portray obvious UV-B targets in their recognition sequences- *Bam* HI and *Eco* RV, (Fig. 2). Enzymes selected on the basis of being potential UV-B targets did not show any differences (*Dra* I, *Eco* RI and *Hind* III). The observed polymorphisms may be attributed to evolutionary processes acting on this natural population possibly resulting in re-arrangements of the genome. This is in agreement with previous reports that chloroplast genomes usually undergo re-arrangements when subjected to stress [8, 14].

Fig. 3 shows chloroplast DNA from different latitudes digested with the *Eco*R V endonuclease and resolved on a 0.8% agarose gel. This is a composite of

ctDNA digests of samples from Augrabies Falls (Lane 1), Bitterfontein (Lane 2) and Kirstenbosch Botanical Gardens (Lane 3). Samples were resolved on the same gel from which a composite was made for comparison purposes and for clarity. λ = λ -*Pst* I molecular weight marker, M = high molecular weight marker IV.

Assessing UV-B radiation sensitivity in plants is not easy. This is because sensitivity differs between species and even varieties and is further influenced by other environmental conditions as well as the developmental history of the plants and geographical origin of the species. It has been hypothesised that species originating from areas that receive high levels of UV-B radiation would be highly resistant to UV-B radiation and there is evidence that species and ecotypes native to low latitudes are inherently more resistant to UV-B irradiation [15]. Since the UV component of sunlight is capable of inducing photo-damage in DNA, plants from areas with high UV-B levels must possess means to prevent DNA damage and repair UV-induced lesions that invariably occur [16]. The absence of any UV-B based differences between the samples in this study could possibly point to the presence of an efficient repair mechanism for UV damaged lesions in *D. sinuata*. It is clear from the results that to fully address this issue, a far more extensive analysis is required. The results do however, indicate the potential for this approach and provide some useful insight into the complexities involved in stress responses in plants. In addition, UV-irradiation is probably a major contributor to plastome damage, but since the chloroplast contains DNA that is used as a transcriptional template for gene products essential for photosynthesis and, therefore plant growth and productivity, it is reasonable to assume that there is an efficient mode of DNA damage repair in the organelles of the Namaqualand daisy, *D. sinuata* at all sites. The structure and expression of the chloroplast genome has been studied in a number of plants [16] and the gene content and the sequence of many genes in the chloroplast have been found to be relatively conserved among land plants. However, an analysis of the entire chloroplast genome of *D. sinuata* has revealed that this is not always the case as evidenced by the polymorphic bands in Fig. 2.

To understand the process of chloroplast genome evolution, information on repeated sequences, intergenic regions and pseudo genes in chloroplast DNA is extremely helpful. Knowledge of the plants' UV-B sensitivity would also shed light on the genetic structure of the different populations and the likely existence of heterogeneity of plants in the different populations and the existence of distinct geographic

patterning of the populations. Future studies could also focus on determining the level of polymorphism between ecotypes of other species growing in the same environments and this would shed some light on the sensitivities of those particular plants to UV-B.

To further enhance the relevance/significance of the results obtained, future studies should look at differences in the sensitivity to UV-B between the samples and identifying a few plants of different sensitivities and limiting studies to those. From the results, it was concluded that there is a difference between the various samples which could be attributed to isolation due to the physical environment. Bitterfontein is physically isolated by the Gamiesberg mountain range and experiences a maritime climate that is foggy most of the time and hence experiences less UV-B levels. On the other hand, Augrabies Falls is in the heart of the Karoo with very clear skies leading to more UV-B radiation. These may be true but unless the differences in sensitivity are very great, it would always be difficult to try and elucidate the mechanism behind the difference based on a limited analysis of chloroplast DNA.

ACKNOWLEDGEMENTS

This study was made possible by grants to S.W. Mpoloka from the Carnegie Corporation of New York, the Rockefeller Foundation, the Ridgefield Foundation and the Coca Cola Foundation through the USHEPIA programme and the University of Botswana.

REFERENCES

1. Green AES, 1983. The penetration of ultraviolet radiation to the ground. *Physiologia Plantarum*, 58: 351-359.
2. Musil, C.F., M.C. Rutherford, L.W. Powrie, L.F. Bjorn and D.J. McDonald, 1999. Spatial and temporal changes in South African solar ultraviolet-B exposure: Implications for threatened taxa. *Ambio*, 28: 450-456.
3. Caldwell, M., R. Robberecht and W.D. Billings, 1980. Steep latitudinal gradient of solar UV-B radiation in the arctic-alpine life zone. *Ecology*, 61: 600-611.
4. Ziska, L.H., A.H. Teramura and J.H. Sullivan, 1992. Physiological sensitivities of plants along an elevational gradient to UV-B radiation. *Am. J. Bot.*, 79: 863-871.
5. Cullis, C.A., S. Swami and Y. Song, 1999. RAPD polymorphisms detected among the flax genotypes. *Plant Mol. Biol.*, 41: 795-800.
6. Hoffer, P.H. and D.A. Christopher, 1997. Structure and blue-light responsive transcription of a chloroplast psbD promoter from *Arabidopsis thaliana*. *Plant Physiol.*, 115: 213-222.
7. Lidholm, J. and Gustafsson, 1991. The chloroplast genome of the gymnosperm *Pinus contorta*: A physical map and a complete collection of overlapping clones. *Current Genet.*, 20: 161-166.
8. Midgley, G.F., S.J.E. Wand and C.F. Musil, 1998. Repeated exposure to enhanced UV-B radiation in successive generations increases developmental instability (fluctuating asymmetry) in a desert annual. *Plant, Cell and Environment*, 21: 437-442.
9. Musil, C.F., 1996. Cumulative effect of elevated ultraviolet-B radiation over three generations of the arid environment ephemeral *Dimorphotheca sinuata* DC (Asteraceae). *Plant Cell and Environment*, 19: 1017-1027.
10. Musil, C.F., 1995. Differential effects of elevated ultraviolet-B radiation on the photochemical and reproductive performances of dicotyledonous and monocotyledonous arid-environment ephemerals. *Plant, Cell and Environment*, 18: 844-854.
11. Waters, E.R. and B.A. Schaal, 1996. Heat shock induces a loss of rRNA-encoding DNA repeats in *Brassica nigra*. *Proc. Natl. Acad. Sci., USA*, 93: 1449-1452.
12. Palmer, J.D., 1986. Isolation and structural analysis of chloroplast DNA. *Methods in Enzymology* 118: 167-186.
13. Musil, C.F. and S.J.E. Wand, 1994. Differential stimulation of an arid-environment winter ephemeral *Dimorphotheca pluvialis* (L.) Moench by ultraviolet-B radiation under nutrient limitation. *Plant, Cell and Environment*, 17: 245-255.
14. Ries, G., W. Heller, H. Puchta, H. Sandermann, H.K. Seidlitz and B. Hohn, 2000. Elevated UV-B radiation reduces genome stability in plants. *Nature*, 406: 98-101.
15. Caldwell, M.M., A.H. Teramura and M. Tevini, 1989. The changing solar ultraviolet climate and the ecological consequences for higher plants. *Tree*, 4: 363-367.
16. Cannon, G.C., L.A. Hedrick and S. Heinhorst, 1995. Repair mechanisms of UV-induced DNA damage in soybean chloroplasts. *Plant Mol. Biol.*, 29: 1267-1277.
17. Wakasugi, T., T. Nagai, M. Kapoor, M. Sugita, M. Ito, S. Ito, J. Tsudzuki, K. Nakashima, T. Tsudzuki, Y. Suzuki, A. Hamada, T. Ohta, A. Inamura, K. Yoshinaga and M. Sugiura, 1997. Complete nucleotide sequence of the chloroplast genome from the green alga *Chlorella vulgaris*: The existence of genes possibly involved in chloroplast division. *Proc. Natl. Acad. Sci., USA*, 94: 5967-5972.

Energy Savings with Variable-Depth Tillage "A Precision Farming Practice"

¹Reza Alimardani, ²Yousef Abbaspour-Gilandeh, ³Ahmad Khalilian,
¹Alireza keyhani and ⁴Seyed Hossein Sadati

¹Department of Agricultural Machinery, Biosystem Engineering College, University of Tehran, Karaj, Iran

²Department of Agricultural Machinery, College of Agriculture,
University of Mohagegh Ardabili, Ardabil, Iran

³Department of Agriculture and Biosystem Engineering, Clemson University,
Edisto Research and Education Center, Blackville, SC 29817

⁴Department of Mechanical Engineering, KNT University of Technology, Tehran, Iran

Abstract: Soil compaction management in the southeastern Coastal Plain soils relies heavily on the use of costly annual deep tillage operations. Variable-depth or site-specific tillage which modifies the physical properties of soil only where the tillage is needed for crop growth, has potential to reduce costs, labor, fuel and energy requirements. Although technology for site-specific tillage is available, there is very limited information on the fuel and energy requirements of site-specific tillage in southeastern coastal plain soils. Tests were carried out on three different coastal plain soils to compare energy requirement of site-specific tillage with uniform-depth tillage operations. Also, the effects of tractor speed, soil texture, moisture contents and electrical conductivity on energy requirement and fuel consumption were determined. The energy saving of 50% and fuel saving of 30% were achieved by site-specific tillage as compared to uniform-depth tillage in a loamy sand soil type. Although draft force increased with an increase in travel speed in all soil types but the tillage depth had bigger effect on the draft and drawbar power than the tractor speed. The effect of soil moisture content on draft force and fuel consumption was not significant in loamy sand and sandy loam soil types. Soil EC was highly correlated to soil texture ($R^2=0.916$) and draft force across the field.

Key words: Tillage energy % precision agriculture % site-specific tillage % tractor speed % soil moisture % electrical conductivity

INTRODUCTION

Soil Compaction is an important problem in the Coastal Plain region. It restricts the root growth into deeper soil layers that are rich in terms of soil moisture and nutrients. Most soils of the southeastern Coastal Plain have a compacted zone or hardpan about 6 to 14 in deep and 2 to 6 in thick. Farmers in this region rely heavily on the use of annual uniform-depth deep tillage to manage soil compaction which improves yields [1, 2]. However, farmers usually do not know if annual subsoiling is required, where it is required in a field, nor the required depth of subsoiling. In addition, there is a great amount of variability in depth and thickness of hardpan layers from field to field and also within the field [3-6]. There is very little to gain from tilling deeper than the compacted layer

and in some cases it may be detrimental to till into the deep clay layer [1]. Applying uniform-depth tillage over the entire field may be either too shallow or too deep and can be costly.

A high-energy input is required to disrupt hardpan layer to promote improved root development and increased drought tolerance. Significant savings in tillage energy could be achieved by site-specific management of soil compaction. Site-specific variable-depth tillage system can be defined as any tillage system which modifies the physical properties of soil only where the tillage is needed for crop growth objectives. Raper [7] estimated that the energy cost of subsoiling can be decreased by as much as 34% with site-specific tillage as compared to the uniform-depth tillage technique currently employed by farmers. Also, Fulton *et al.* [8] reported

a 50% reduction in fuel consumption by site-specific or precision deep tillage.

Tillage implement energy is directly related to working depth, tool geometry, travel speed, width of the implement and soil properties [9, 10]. Soil properties that contribute to tillage energy are moisture content, bulk density, cone index and soil texture [11]. It has been reported that draft on tillage tools increases significantly with speed and the relationship varies from linear to quadratic. Similarly, effect of depth on draft, also varies linearly [12].

The technology for site-specific tillage (variable depth tillage) is available [13] and the concept of site-specific tillage has been studied by some researchers [6, 7]. However, this is an emerging technology and therefore minimal information is available on draft and energy requirements of variable-depth tillage, an important consideration in selecting tillage systems. Furthermore, there is a need to determine the effects of tractor speed and soil parameters such as texture, moisture and electrical conductivity on energy requirements of site-specific and conventional uniform-depth tillage operations in coastal plain soils. The development of this information is the prime concern for an economical management of soil compaction and adoption of this technology by southeastern farmers.

The objectives of this study were:

- C To compare the energy requirement and fuel consumption between site-specific tillage and uniform-depth tillage on three different coastal plain soils.
- C To determine the effects of tractor speed and soil parameters such as texture, moisture and electrical conductivity on tillage energy requirements and tractor fuel consumption.

MATERIALS AND METHODS

Equipment: A commercially available soil electrical conductivity meter, Veris Technologies 3100, was used to map the Electrical Conductivity (EC) of the test field [14]. The system is equipped with six coulter-electrodes. One pair of electrodes applies a current into the soil, while others measure the voltage drop between the coulters. The system can measure the EC in either the top 30 or 90 cm of soil.

A DGPS-based penetrometer system mounted on a John Deere Gator was used to quantify geo-referenced soil resistance to penetration [13]. The driver of the Gator could operate the penetrometer (Fig. 1). Soil cone index values were calculated from the measured force required pushing a 130-mm² base area, 30-degree cone into the soil [15].

A front-wheel-assist, 78.3 kW (105 HP) instrumented tractor (John Deere 4050) was used to collect the energy consumption data during the tillage operations. The instrumentation system consisted of a three-point-hitch dynamometer, a fuel flow meter, engine speed (RPM) sensor, several ground speed sensors (fifth wheel, radar and ultrasonic), Differential Geographical Positioning System (DGPS) unit, a data logger and an optical sensor determining the start and end of each plot [6].

DGPS-based equipment for controlling the tillage depth to match soil physical parameters was used in this experiment (Fig. 2). This equipment can control the tillage depth "on-the go" using either a soil compaction map, inputs from an instrumented shank, or entering the tillage depth data manually in the computer [13]. The two outside shanks of a 4-row subsoiler were removed for the tillage energy requirement study.

Field test: Field experiments were carried out, on coastal plain soils, in the fall of 2004 at the Edisto Research and Education Center of Clemson University near Blackville, South Carolina (Latitude 33°21"N, Longitude 81°18"W). The 6-acre test field had three different soil types: Faceville loamy sand, Fuquay sandy loam and Lakeland sand.

Prior to initiation of tests, EC measurements were obtained with the Veris unit to determine variations in soil texture and soil physical properties across the field. A geo-referenced EC map was developed using SStoolbox GIS software. The results showed a great amount of variability in soil EC and the field was found to be an ideal site for variable-depth tillage study. The test field was then divided into 12.5×50 ft rectangular plots and soil samples were collected from each plot and analyzed for soil texture. Figure 3 shows soil electrical conductivity map, soil types and plot arrangements over the entire field.

A complete set of cone penetrometer measurements were obtained with the DGPS-based penetrometer system across the entire field. Nine geo-referenced penetrometer measurements, 5 ft apart, were taken from each plot. The



Fig. 1: Hydraulically operated penetrometer system with DGPS unit



Fig. 2: The control system for variable-depth tillage operations

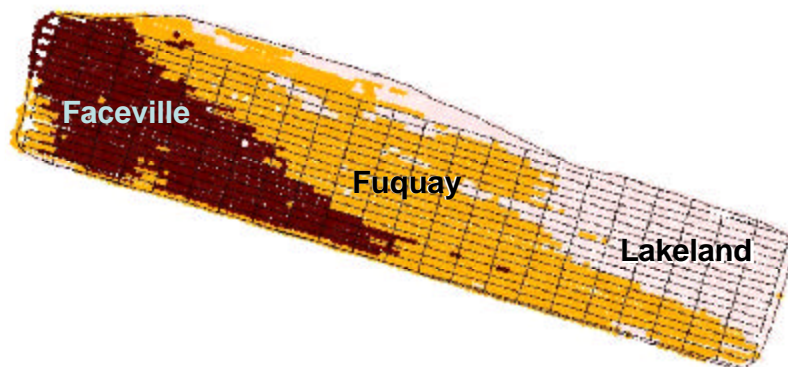


Fig. 3: Aerial photograph and soil electrical conductivity map of the experimental field

depth and thickness of the hardpan were determined from the collected data using the criteria defined by Taylor and Gardener [16]. Within each plot, it was decided to set the tillage depth that would rupture compacted layers of the soil with cone index values above 300 psi.

Tillage experiments consisted of twelve treatments arranged in randomized complete blocks with three replications in each soil type. The treatments included two tillage systems (site-specific and uniform-depth), three levels of tractor speed (4, 5 and 6 mile/h) and two levels of soil moisture contents.

RESULTS AND DISCUSSION

The penetrometer data in each location was analyzed using an algorithm written in QBASIC program [6] for determining the tillage depth. A single depth-value was assigned to each plot by averaging the nine predicted-tillage-depth values within that particular plot. Using these data three tillage zones were identified in each soil type. In each zone, the two tillage treatments (uniform-depth and site-specific) were replicated 3 times.

The uniform-depth tillage was performed 18 in deep to completely disrupt the root-impeding layer. The site-specific tillage was applied according to the application maps generated from soil compaction data. The predicted tillage depth in Faceville soil type ranged from 8 to 14 in. In both Fuquay and Lakeland soil types, the tillage depth varied from 11 in to 18 in

Statistical analysis of energy requirement by using Proc ANOVA in SAS software [17] clearly showed significant difference between tillage treatments in every soil types ($p < 0.01$). Also fuel consumption was significantly different in Faceville soil ($p < 0.01$) and also in the other two soil types ($p < 0.05$) between site-specific and uniform-depth tillage.

Comparison of tillage energy and fuel consumption for both tillage systems in Faceville soil type showed that energy saving of 50% and fuel saving of 30% could be achieved by using site-specific tillage system. Also energy and fuel savings were 21 and 8% for Fuquay and 26.1 and 8.5% Lakeland soil type respectively. Figure 4 shows the energy requirements and fuel consumption for both tillage systems in each soil type.

Although not statistically different, the draft force increased with an increase in tractor speed in all soil types. Also the results showed a strong correlation between the tractor speed and fuel consumption (gal/acre) in each soil types. This is due to increase in draft force

and consequently increase in drawbar power. However, the tillage depth had bigger effect on the draft and drawbar power than the tractor speed.

The effect of moisture content on draft force and fuel consumption was not significant at loamy sand (Faceville) and sandy loam (Fuquay) soil types. However, an increase in soil moisture content resulted in a decrease in draft forces and fuel consumptions. In sandy soil type (Lakeland), draft forces and fuel consumptions decreased significantly when soil moisture content increased. This could be due to significant changes in cone index values, since only in this soil type cone index values were significantly affected by soil moisture contents compared to other soil types.

Results showed that use of soil electrical conductivity (soil EC) to predict soil texture and tillage draft requirement was very successful. There was strong linear correlation between soil EC and both soil texture and tillage draft requirement at a given depth and speed. This indicates that draft requirement strongly vary with soil texture and depends on clay and sand content of soil. Also for practical applications, EC data can be used to predict areas of the field with high or low tillage draft requirements. The Veris system provided reading from 0.1 to 7.0 mS mG^{-1} , predicting percentage of clay across the field with a linear correlation coefficient of 0.912 and percentage of sand with a correlation coefficient of 0.916. Figure 5 shows the effects of soil texture (%clay) on soil electrical conductivity. A portion of the draft-requirement data with the same tillage depth (18 in) was selected to investigate the correlation between draft and soil EC. There was a very strong correlation between EC data and tillage draft force at a given speed. Figure 6 shows the effects of EC data on draft force at three different speeds that have been obtained within three different soil types.

CONCLUSIONS

The site-specific tillage resulted in a considerable energy saving of 50% and fuel saving of 30% in loamy sand soil type compared to conventional uniform-depth tillage. Also, energy and fuel savings were 21 and 8% for sandy loam and 26.1 and 8.5% for sandy soil type respectively.

The draft force increased as the travel speed increased in all soil types. However, the tillage depth had bigger effect on the draft and drawbar power than the tractor speed.

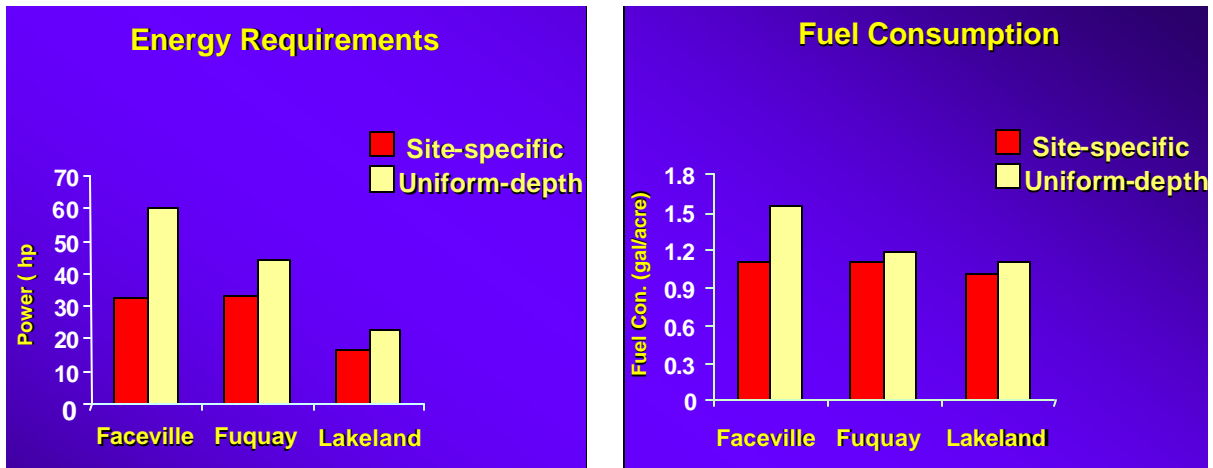


Fig. 4: Energy requirements and fuel consumption for site-specific and uniform-depth tillage

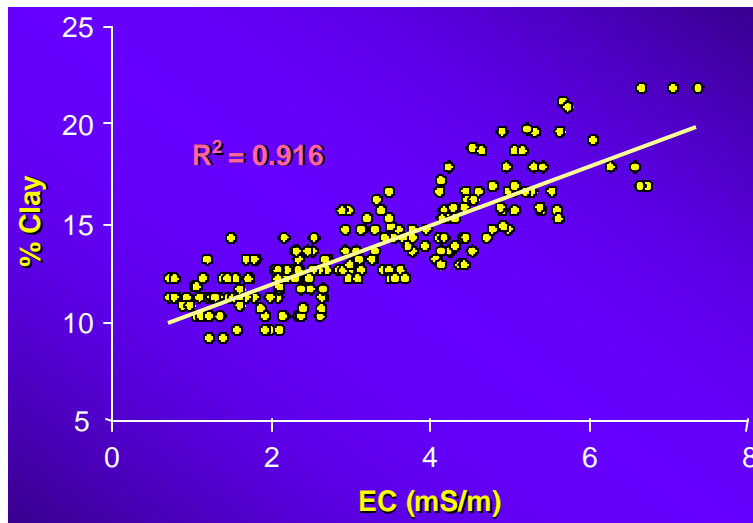


Fig. 5: Effect of soil texture (percentage of clay) on soil electrical conductivity

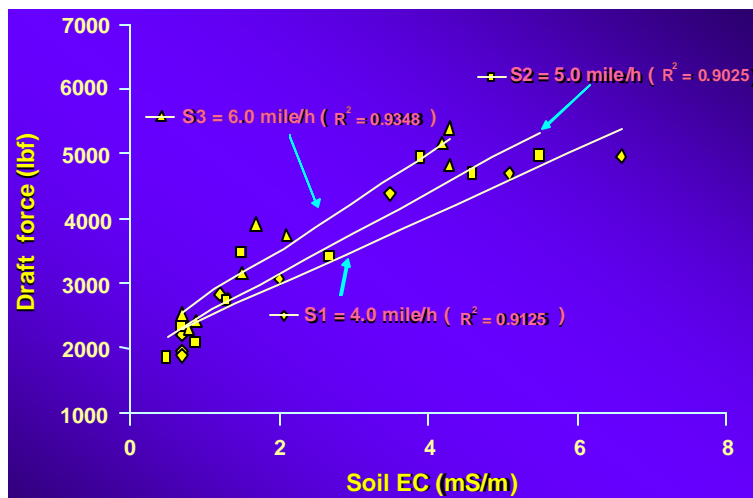


Fig. 6: Effect of soil electrical conductivity on draft force

The effect of soil moisture content on draft force and fuel consumption was not significant in loamy sand and sandy loam soil types. However, draft force and fuel consumption had a negative correlation with the soil moisture contents.

Soil EC data were highly correlated to soil texture (%clay content) with a correlation coefficient of 0.916.

There was a strong linear correlation between soil electrical conductivity and draft force across the field.

REFERENCES

1. Garner, T.H., A. Khalilian and M.J. Sullivan, 1989. Deep tillage for cotton in Coastal Plain soils costs/returns. In the Proceedings of 1989 Beltwide Cotton Production Research Conferences, Nashville, TN, pp: 168-171.
2. Khalilian, A., Michael Jones, Mike Sullivan and James Frederick, 2004. Comparison of strip tillage system in coastal plain soils for cotton production. In the Proceedings of the 2004 Beltwide Cotton Conferences, National Cotton Council of America, Memphis, TN.
3. Raper, R.L., E.B. Schwab and S.M. Dabney, 2000a. Spatial variation of the depth of root restricting layers in Northern Mississippi soils. In: Proceeding of the 2000 2nd Intl. Conf. Geospatial Information in Agriculture and Forestry, Lake Buena Vista, FL, 1: 249-256.
4. Raper, R.L., E.B. Schwab and S.M. Dabney, 2000b. Site-specific measurement of site-specific compaction in the Southeastern United States. In the Proceedings of the 2000, 15th ISTRO Conference, Ft. Worth, TX, July 3-7.
5. Clark, R.L., 1999. Evaluation of the potential to develop soil strength maps using a cone penetrometer. Presented at the 1999 ASAE Annual International Meeting, Paper No.99-3109, ASAE, 2950 Niles Road, St. Joseph, MI., USA.
6. Gorucu, S., A. Khalilian, Y.J. Han, R.B. Dodd, F.J. Wolak and M. Keskin, 2001. Variable depth tillage based on geo-referenced soil compaction data in Coastal Plain region of South Carolina. ASAE Paper No. 011016. St. Joseph, MI., USA.
7. Raper, R.L., 1999. Site-specific tillage for site-specific compaction: Is there a need? In the Proceedings of the 1999 International Conference of Dryland Conservation/Zone Tillage, Beijing, China.
8. Fulton, J.P., L.G. Wells, S.A. Shearer and R.I. Barnhisel, 1996. Spatial variation of soil physical properties: A precursor to precision tillage. Presented at the 1996 ASAE Annual International Meeting, Paper No.96-1012, ASAE, 2950 Niles Road, St. Joseph, MI, USA.
9. Gill, W.R. and G.E. Vanden Berg, 1968. Soil dynamics in tillage and traction. Agricultural handbook. Washington DC: USDA-Agric. Res. Service, pp: 316.
10. Palmer, A.L. and I.R. Kruger, 1982. Comparative drafts of six tillage implements. In the Proceeding of the 1982 Conference on Agricultural Engineering,, Armidale, NSW Australia, 22-24 August. Barton, Australia, pp: 163-167.
11. Upadhyaya, S.K., T.H. Williams, L.J. Kemble and N.E. Collins, 1984. Energy requirement for chiseling in coastal plain soils. Transactions of the ASAE, 36: 1267-1270.
12. Al-Janobi, A.A. and S.A. Al-Suhaibani, 1998. Draft of primary tillage implements in sandy loam soil. Applied Eng. Agric., 14: 343-348.
13. Khalilian, A., Y.J. Han, R.B. Dodd, Mike J. Sullivan, S. Gorucu and M. Keskin, 2002. A control system for variable depth tillage. ASAE Paper No. 021209. St. Joseph, MI, USA.
14. Lund, E.D., C.D. Christy and P.E. Drummond, 1999. Practical applications of soil electrical conductivity mapping. In the Proceeding of the 1999 J.V. Stafford (Ed.) Precision Agriculture of the 2nd Eur. Conf. on Precision Agriculture. Denmark, 11-15 July 1999. SCI, Sheffield, UK, pp: 771-779.
15. ASAE Standards, 2004. ASAE S313.3 FEB04: Soil cone penetrometer. In: Hahn, R.H., M.A. Purschwitz and E.E. Rosentreter (Eds.). ASAE Standards 2004. ASAE, St. Joseph, MI.
16. Taylor, H.M and H.R. Gardner, 1963. Penetration of cotton seedlings taproots as influenced by bulk density, moisture content and strength of soil. Soil Sci., 96: 153-156.
17. SAS Institute Inc., 1999. SAS/STAT® User's Guide, Version 8, Cary, NC: SAS Institute Inc.

Comparisons of Object-Oriented and Pixel-Based Classification of Land Use/Land Cover Types Based on Landsat7, Etm⁺ Spectral Bands (Case Study: Arid Region of Iran)

¹H.R. Matinfar, ²F. Sarmadian ³S.K. Alavi Panah and ⁴R.J. Heck

¹University of Lorestan, Iran

²Department of Soil Science, University of Tehran, Iran

³College of Geography, University of Tehran, Iran

⁴Land Resource Science Department, University of Guelph, Iran

Abstract: In this study, land cover types of Kashan test area were analyzed on the basis of the classification results acquired using the pixel-based and object-based image analysis approaches. Landsat7 (ETM⁺) with six spectral bands was used to carry out the image classification and ground truth data were collected from available maps (Soil and Saline soil maps, Topographic map and Geological map), field observation and personal knowledge. In pixel-based image analysis supervised classification was performed using the Minimum distance through Geomatica V.9.1. On the other hand, object-oriented image analysis was evaluated through eCognition software. During the implementation, several different sets of parameters were tested for image segmentation and standard nearest neighbor was used as the classifier. The results of classified images have shown that the object-oriented approach gave more accurate results, (Including higher producer's and user's accuracy for most of the land cover classes) in the studied arid region than those achieved by pixel-based classification algorithms.

Key words: Land cover · multispectral segmentation · classification · landsat7 · remote sensing

INTRODUCTION

Earth observation from space so called remote sensing, offers powerful capabilities for understanding, forecasting, managing and decision making about our planet's resources. Remotely sensed image data from earth observation sensor systems is widely used in a range of terrestrial and atmospheric application, such as land cover mapping, environmental modeling and monitoring and the updating of geographical databases. For these applications, remote sensing methods and techniques have been proved to be a very useful tool and usually a thematic map is required [2, 9]. A thematic map displays the spatial variation of a specified phenomenon, such as land cover type, soil type or vegetation type. The trustworthiness and reliability of these thematic maps depend on how we analyze remotely sensed images.

Remotely sensed image analysis is a challenging task. One popular and commonly used approach to image analysis is digital image classification. The purpose of image classification is to label the pixels in the image with meaningful information of the real world [8]. Through classification of digital remote sensing image, thematic maps bearing the information

such as the land cover type; vegetation type etc. can be obtained [13].

In this study there are two-classification approaches selected. One is traditional pixel based image analysis approach and the other one is the object-oriented image analysis approach.

Typical method of classification of remote sensing imagery has been pixel based. Normally, multispectral data are used to perform the classification and, indeed the spectral pattern present within the data for each pixel is used as the numerical basis for categorization. That is different feature types with different combination [10, 12]. Pixel based approach is based on conventional statistical techniques, such as supervised and unsupervised classification. In supervised classification the image analyzed "supervised" the pixel categorization process by specifying, to the computer algorithm, numerical description of the various land cover types present in a scene. In order to this representative sample sites of known cover type, called training area are used to compile a numerical "interpretation key" that describes the spectral attributes for each feature type of interest. Each pixel in the data set is then compared numerically to each category in

the interpretation key and labeled with the name of the category it looks more like.

Recently, considerable advancements have been made in the development of object-based, object oriented image analysis approach is the approach to image analysis combine spectral information and spatial information, so with object oriented image analysis approach not only the spectral information in the image will be used as classification information, the texture and context information in the image will be combined into classification as well [4]. The image will be segmented into objects that form the classification units and will be treated as a whole in the classification process. Object oriented image classification approach is based on fuzzy theory, in which an object will be classified into more than one class with different membership values [11]

The most evident difference between pixel-based and object-based image analyses is that first, in object oriented image analysis; the basic processing units are image objects or segments, not single pixels. Second, the classification in object oriented image analysis are soft classifies that is based on fuzzy logic. Soft classifies use membership to express an object's assignment to a class. The membership value usually lies between 1.0 and 0.0 where 1.0 expresses a complete assignment to a class and 0.0 expresses absolutely improbability. The degree of membership depend on the degree to which the objects fulfill the class-describing condition. One advantage of these soft classifications lays their possibility to express uncertainties about the classes' descriptions. And finally unlike pixel-based classification, the object oriented approaches as output a thematic map composed of geographical entities labeled with land cover classes and, as much, can be directly sorted into GIS databases, creating or updating usable geo information [5, 6].

The spectral and also spatial resolution of 30 m of Landsat Enhanced Thematic Mapper (ETM⁺) data are important characteristics for land use/land cover mapping. Ideally the spectral response should be homogenous within the land cover unit boundary and different from adjacent units, research shows that Landsat bands have a good potential for responding to the differences in land cover properties and hence the separation of land cover types. Also ETM⁺ data is the cheapest and available remote sensing data for Kashan area that we can use for test on classification of arid region land cover/land use types.

The main objective of this study is to compare pixel-based and object-based techniques for the classification of LANDSAT 7(ETM⁺) imagery of kashan Area of Iran, according to land cover and land use.

MATERIALS AND METHODS

The research was carried out in the Kashan area located in the central Kavir of Iran (Fig. 1).

Geographical coordinates of the area between 33°45' to 34°45' N and 51° to 51° 30' E. The study site covers an area of approximately 90000 ha. The area has an arid climate, with cold winter and hot dry summer, the amount of annual rainfall is 139 mm, most of the precipitation falls in spring and winter. The mean annual, the summer maximum and the winter minimum temperatures are 19.46°C, 42.51°C and -1.97°C respectively. The soil temperature regime is thermic and the soil moisture regime is aridic and approximately level to undulating topography area.

Cloud-free ETM⁺ data, collected Aug. 9, 2002 were used in this study, summer data provide a large proportion of bare soil and a minimum of vegetation in poor range and sparse farms area where cereal is a major crop. According to the Landsat World Reference

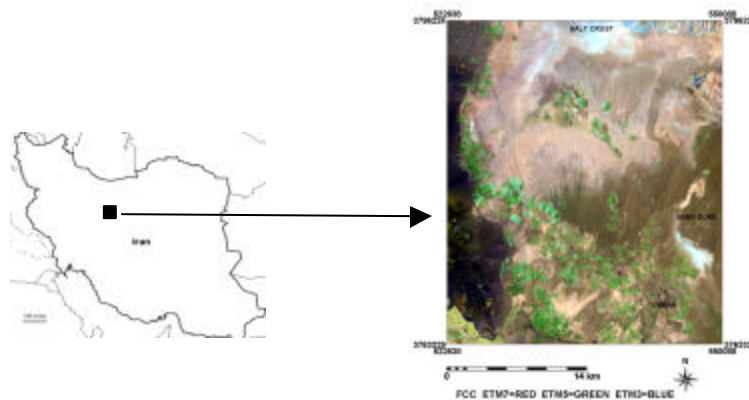


Fig. 1: The study area-Kashan-Iran

Table 1: Landsat7 and ETM⁺ characteristics

Band number	Spectral range (micron)	Ground resolution (m)
1	0.45 to 0.515	30
2	0.525 to 0.605	30
3	0.63 to 0.690	30
4	0.75 to 0.90	30
5	1.55 to 1.75	30
6	10.40 to 12.5	60
7	2.09 to 2.35	30
8	0.52 to 0.9	15
Swath width	185 Kilometers	
Repeat coverage interval	16 days (233 orbits)	
Altitude	705 Kilometers	
Quantization	Best 8 of 9 Bits	
Inclination	Sun-synchronous, 98.2 degrees	

System (WRS), the satellite image for the study area is located at Path 164, Row 36; its ID is 080021001002100034 and was obtained from the sensors on board of Landsat7.

The ground truth data were collected from field observation, personal knowledge and; Soil and saline soil maps of Abshirin and Aran area 1:50000 scale.

Geological map of Aran area 1:100000 scale and Topographic maps of Abshirin and Aran area 1:50000 scale.

Image processing: The images were georeferenced using UTM map projection for zone 39 and datum of WGS84. The images were resampled to 28.5 m for 1, 2, 3,4,5,7 bands, 14.25 m for panchromatic and 57 m for thermal bands per pixel, using the nearest neighbor technique. The subarea of 967*1157 pixels were extracted for a more detailed comparative analysis. In order to produce test area, false color composite from ETM⁺ bands of 7,5 and 3 were used, while all of the six bands (ETM⁺ 1,2,3,4,5 and7 bands) were used for classification by two methods object-based and pixel-based.

Accuracy assessment: Another area that is continuing to receive increased attention by remote sensing specialists is that of classification accuracy [10]. eCognition supplies a method to assess the accuracy by error matrix based on test areas (ground truth). By defining the ground truth mask, eCognition generates an error matrix automatically. In order to compare the accuracy of the classification result created by the two approaches, pixel-based and object-based, the same set of ground truth was used; here the classification image created in eCognition was exported into Geomatica V.9.1 software. In Geomatica the classified images were

crossed with the ground truth map (test area) to perform the error matrix (Table 3 and 5).

Pixel-base classification: Supervised classification was performed using ETM⁺ bands. In supervised classification, the basic steps followed are (1) select training samples which are representative and typical for that information class; (2) perform classification after specifying the training samples set and classification algorithms;(3) assess the accuracy of the classified image through analysis of a confusion matrix which is generated either through random sampling or using test areas as reference data [7]. ILWIS academic version 3.2 was used for minimum distance classification, test area production and accuracy assessment.

Training samples are selected according to the ground truth. These homogenous areas are identified in the image to form the training samples for all of the information classes. The selected algorithm for performing the supervised classification is the minimum distance classification. In this algorithm first the mean spectral value in each band for each class is determined. These values comprise the mean vector for each class. A pixel of unknown identity may be classified by computing the distance between the value of the unknown pixel and each of the class means, if the pixel were further than an analyst defined distance (distance threshold) from any class mean, it would be classified as “unknown” [10]. This distance threshold could vary for each class depending on the expected degree of compactness of that class. Compactness might be estimated from the standard deviation for each feature of the pixels making up the training sample for a given class.

Object-base classification: eCognition professional version 4.0 was used for object-oriented analysis and classification.

Segmentation is the main process in the eCognition software and its aim is to create meaningful objects. This means that an image object should ideally represent the shape of each object in question. This shape combined with further derivative color and texture properties can be used to initially classify the image by classifying the generated image objects. Thereby the classes are organized within a class hierarchy. With respect to the multi-scale behavior of the objects to detect a number of small objects can be aggregated to form larger objects constructing a semantic hierarchy. In performing the segmentation of ETM⁺, six spectral bands (ETM⁺1, 2, 3, 4, 5&7) took in the segmentation process with a full weight (1.0) [1, 3].

Table 2: Segmentation parameters used for image

Segmentation level in object hierarchy	Scale parameter	Homogeneity criterion		Shape criterion		Segmentation mode
		Color	Shape	Smoothness	Compactness	
1	10	0.2	0.8	0.1	0.9	Normal
2	17	0.6	0.4	0.6	0.4	Normal
3	20	0.5	0.5	0.5	0.5	Normal

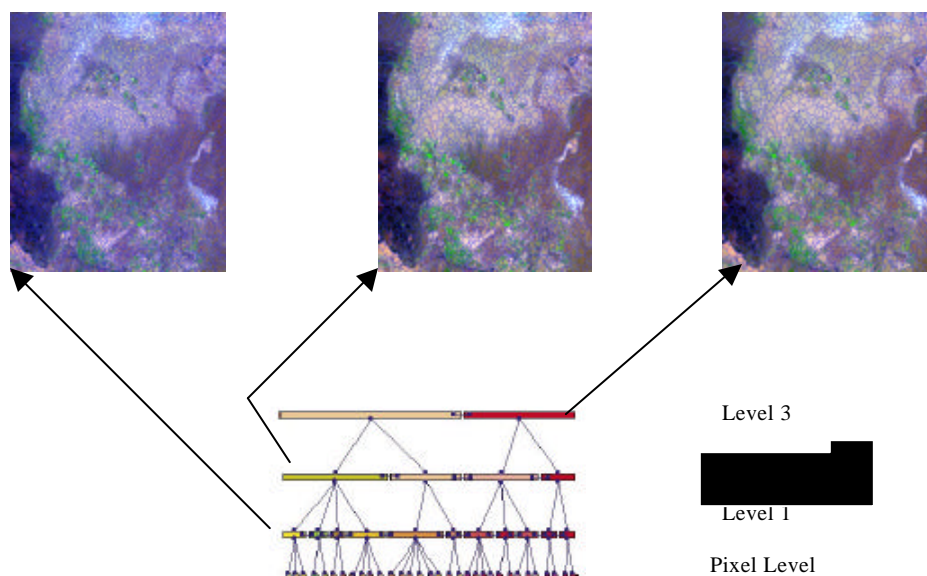


Fig. 2: Hierarchical net of image objects derived from image segmentation, level 1(scale parameter 10), Level 2 (scale parameter 17), level 3(scale parameter 20)

Object-base segmentation was tried using different scale parameters (Table 2). As can be realized that the smaller scale parameter increases the dimensionality and dividing the object into the sub-groups while the larger scale combines the multi segment into one. By testing different segmentation parameters, finally according visual comparison and ground truth and personal knowledge a set of segmentation parameters were selected. Based on these parameters, segmentation process is performed (Fig. 2)

After deciding how many classes need to be distinguished, considering the image classification objective, class name and class color need to be identified through classification are, Agriculture (Agr.), Alluvial fan (Al.), Desert crust (DC.), Non saline soil (NS-S), Orchard (Or.), Outcrop-igneous (OC-I), Outcrop-limestone (OC-L), piedmont (Pi), Rural (Ru.), Saline soil (SS.), Salt crust (SC.), Sand dune-longitudinal (SD-L), Small sand dune (SS-D.) and Urban (Ur.) After assigning classes, the nearest neighbor algorithm defined as classifier. Using nearest neighbor, as the classifier which is similar to supervised classification and therefore training areas have been

selected. In eCognition the training areas are training objects; one sample object covers many typical pixel samples and their variation. Starting with a few samples and adding only necessary samples in subsequent steps is a very efficient way to come up with a successful classification [3].

RESULTS

Pixel-based and objects oriented image analysis approaches have been performed by classifying the remote sensing image of Landsat ETM⁺. The accuracy of the classification result using these two approaches has also been assessed by creating the error matrix using the same test area as reference data. Comparisons of the results of the accuracy assessment showed that object oriented image analysis attains higher overall accuracy and higher land cover class accuracy (producer's accuracy and user's accuracy) for most of the classified land cover class.

Pixel based classification results: Pixel based image analysis means that the classic image classification

Table 3: Confusion matrix for pixel-based image classification

Classes	Classification result														Producer accuracy
	Agr.	Al.	DC.	NS-S.	Or.	OC-I.	OC-L.	Pi.	Ru.	SS.	Sc.	SD-L.	S-SD.	Ur.	
Test set															
Agr.	352	0	0	7	0	0	7	0	0	12	0	0	0	0	0.93
Al.	0	297	0	0	0	47	0	0	0	0	0	0	0	0	0.86
Dc.	0	0	262	46	0	0	0	0	0	0	0	0	0	0	0.58
NS-S.	0	0	0	117	0	0	0	0	0	0	0	0	0	0	1.0
Or.	0	0	0	0	138	0	0	0	0	0	0	0	0	0	1.0
OC-I.	0	42	0	0	0	680	0	0	0	0	0	0	0	0	0.94
OC-L.	0	0	23	5	0	0	206	0	0	0	0	0	0	0	0.88
Pi.	0	97	0	0	0	1	0	234	0	0	0	0	0	0	0.70
Ru.	0	0	0	0	0	0	0	1	143	19	0	1	26	170	0.40
SS.	0	0	0	0	0	0	0	0	0	275	0	0	0	1	0.99
SC.	0	0	0	0	0	0	0	0	0	0	391	0	0	0	1.0
SD-L.	0	0	0	0	0	0	0	124	0	0	0	422	44	0	0.72
S-SD.	0	0	0	0	0	0	0	0	4	0	0	21	395	0	0.94
Ur.	0	4	0	0	0	0	1	41	92	27	0	5	69	139	0.37
User accuracy	1	0.68	0.92	0.67	1.0	0.93	0.96	0.58	0.6	0.82	1.0	0.94	0.74	0.45	
Overall accuracy = 0.81															

method that classifies remote sensing images according to the spectral information in the image and the classification manner is pixel by pixel and one pixel only belongs to one class.

In supervised classification, the image analyze supervises the pixel categorization process by specifying, to the computer algorithm, numerical descriptors of the various land-cover types present in an image. Training samples that describe the typical spectral pattern of the land cover classes are defined. Pixels in the image are compared numerically to the training samples and are labeled to the land cover class that has similar characteristics. There are three basic steps involved in the supervised classification method: training stage, classification stage and accuracy assessment stage, these three stages are applied in the classification process of ETM⁺ spectral bands.

Landsat7 ETM⁺ with 6 bands (bands1-5 and bands7) was used for supervised classification. Combining the fieldwork survey of the study area and also the image classification objective, the fourteen-information classes needed to be identified by automatic image classification. These information classes are introduced before. Training samples are selected according to the ground truth from fieldwork; these homogenous land cover areas are identified in the image to form the training samples for all of the information classes. The selected algorithm for performing the supervised classification is the

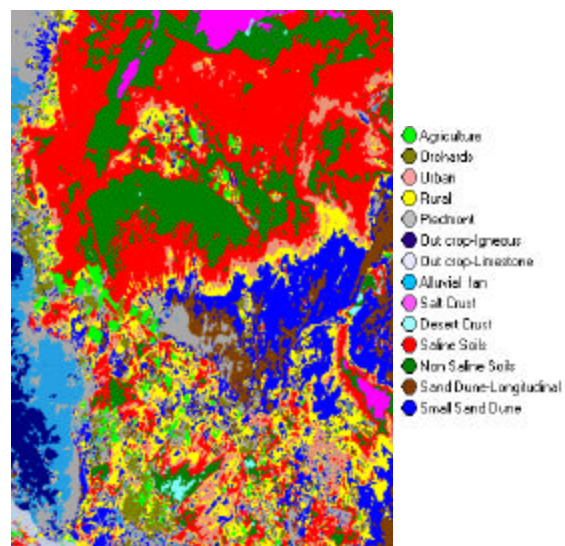


Fig. 3: Pixel-based classification result

minimum distance classifier. Classified image shows distribution of land covers /use types according this algorithm (Fig. 3).

A classification is not complete until its accuracy is assessed [10]. This explains the importance of accuracy assessment of the classification result. Accuracy assessment is a general term for comparing the classification result to the ground truth, in order to determine the accuracy of the classification process.

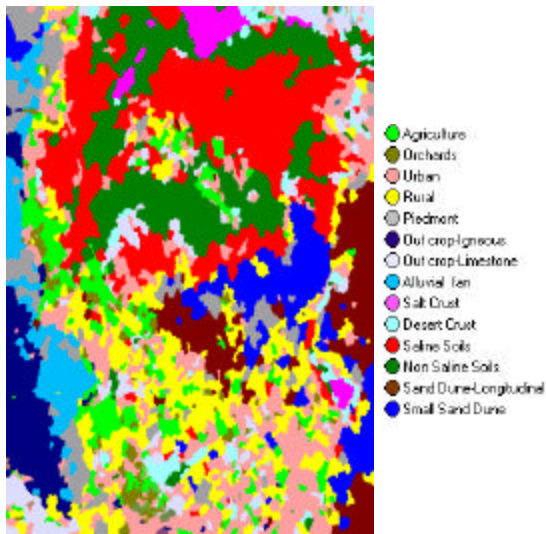


Fig. 4: Object-based classification result

One of the most common methods of expressing the classification accuracy is the preparation of a classification error matrix or confusion matrix.

Accuracy assessment result is given in Table 3 and Table 5. As we see in Table 3 the overall accuracy for pixel base image classification is 81%. Information classes of “salt crust” and “orchard” have both high producer’s and user’s accuracy, but the information classes of “urban” has a low producer’s and user’s accuracy. The reason of this low accuracy is that there is a similarity between the roof of settlement building materials and other land cover such as “rural”, “saline soil”, “piedmont” and “small sand dune”, in their spectral reflectance. By image classified pixels of “urban”, “rural”, “saline soil”, “piedmont” and “small san dune” could be grouped into one object; this could be due to the miss-classification between these five class.

Object base classification results: In eCognition, classified image objects are not only assigned to one class or not, but also get a detailed list with the membership values of each of the class contained in the class hierarchy. An image object is assigned to the class with highest membership value, as long as this highest membership value equals at least the minimum membership value that can be edited. It is significant for the quality of a classification result the highest membership value of an image object is absolutely high, indicating that the image object attributes are well suited to at least one of the class description [3]. Due to eCognition fuzzy classification concept, an image object has memberships in more than one class. The classification with the highest assignment values is taken as the best classification result.

Table 4: Best classification result

Class	Objects	Mean	StdDev	Min	Max
Agriculture	245	0.57	0.20	0.10	1
Orchard	98	0.53	0.21	0.10	1
Urban	458	0.69	0.18	0.10	1
Rural	351	0.83	0.10	0.46	1
Piedmont	94	0.89	0.06	0.73	1
Out crop-igneous	101	0.80	0.20	0.21	1
Out crop-limestone	140	0.62	0.15	0.17	1
Alluvial fan	81	0.91	0.08	0.51	1
Salt crust	45	0.77	0.18	0.24	1
Desert crust	78	0.68	0.19	0.12	1
Saline soils	374	0.89	0.08	0.59	1
Non-saline soils	250	0.87	0.10	0.49	1
Sand dune-longitudinal	109	0.90	0.08	0.68	1
Small sand dune	114	0.93	0.06	0.76	1

Table 4 shows the accuracy assessment for classification results of ETM⁺ data for best classification result and Fig. 4 shows the image classification result. As we can see from this table the best classification value for all land cover classes except for “agriculture” and “orchard” classes are high. The classes mean value and standard deviation show that most of the objects were classified with high membership values.

Accuracy assessment result is given in Table 5. It is the error matrix by test area. From Table 5 it can be seen that the overall accuracy is 91%, considering the producer’s and user’s accuracy of individual class, for the “Agriculture” the producer’s accuracy is 87% and the user’s accuracy is 78%. This means 87 percentage of the agriculture is correctly identified and also 78% of the area that is classified as “agriculture” is truly this category. For the “salt crust” the producer’s and user’s accuracy is 100 and 83% respectively. By the result we can say all of the “salt crust” is correctly identified and 83% of the area that is classified as “salt crust” is truly this category.

Also we can see there are four classes with the lowest producer’s and user’s accuracy. They are information classes “agriculture”, “orchard”, “piedmont” and “Rural”, for the “rural” the producer’s and user’s accuracy are 73% and 83% respectively, for the “piedmont” the user’s accuracy is 80%, for the “agriculture” the user’s accuracy is 78% and for “orchard” the user’s accuracy is 72%. There is significant confusion between “rural” and “urban” and “orchard” and “agriculture” and between “piedmont” and “agriculture” and “sand dune-longitudinal” and between “agriculture” and “orchard”. From field survey it was found that information class “piedmont” mainly

Table 5: Confusion matrix for object-based image classification

Classes	Classification result														Producer accuracy
	Agr.	Al.	DC.	NS-S.	Or.	OC-I.	OC-L.	Pi.	Ru.	SS.	Sc.	SD-L.	S-SD.	Ur.	
Test set															
Agr.	348	0	0	0	20	0	10	10	10	0	0	0	0	0	0.87
Al.	0	307	0	0	0	0	0	0	0	0	0	0	19	0	0.94
Dc.	0	0	274	0	0	0	34	0	0	0	0	0	0	0	0.89
NS-S.	0	0	0	112	0	0	0	0	0	5	0	0	0	0	0.95
Or.	4	0	0	0	134	0	0	0	0	0	0	0	0	0	0.97
OC-I.	0	0	0	0	0	674	0	0	0	0	48	0	0	0	0.93
OC-L.	26	0	0	0	0	0	208	0	0	0	0	0	0	0	0.89
Pi.	0	0	0	0	0	0	0	302	0	0	30	0	0	0	0.91
Ru.	32	0	0	0	32	0	0	0	263	0	0	0	0	33	0.73
SS.	0	0	0	16	0	0	0	0	0	260	0	0	0	0	0.94
SC.	0	0	0	0	0	0	0	0	0	0	391	0	0	0	1.0
SD-L.	0	0	0	0	0	0	0	65	0	0	0	525	0	0	0.89
S-SD.	38	0	0	0	0	0	0	0	0	0	0	0	382	0	0.91
Ur.	0	0	0	0	0	0	0	0	42	0	0	0	0	336	0.89
User accuracy	0.78	1.0	1.0	0.88	0.72	1.0	0.83	0.80	0.83	0.98	0.83	1.0	0.95	0.91	
Overall accuracy = 0.91															

along the agriculture area, by image segmentation pixels, which are located along the boundary of “piedmont” and “agriculture” could be grouped in to the same objects with pixels of “agriculture”. By object-oriented classification pixels that are “agriculture” could be classified as “piedmont” by being in the same object. Also from field survey information class “rural” is characterized by having the mixture “orchard” and “agriculture” and some similarity “urban” information. The reason of confusion between “rural” and “urban” is because approximately the material of these two classes is similar and this causes the spectral information of these two classes to be similar. By image segmentation pixels of “urban” and “rural” could be grouped into one object; this could be due to the miss-classification between these two classes and the reason of the confusion between “rural” and “orchard”, because there are mixed pixels of rural and orchard. So by image segmentation pixels of “rural” and “orchard” could be grouped into one object; this could be case the miss-classification.

Except these four information classes having low producer’s and user’s accuracy; the other information classes have high or relatively high producer’s and user’s accuracy.

DISCUSSION

Pixel-based and object-oriented image analysis approaches have been performed by classifying the

remote sensing image of Landsat7 (ETM⁺). Accuracy of the Classification result using these two approaches has also been assessed by creating the error matrix.

Comparison of the result of the accuracy assessment shows that object oriented image analysis attain higher overall accuracy and higher individual producer’s and user’s accuracy for each classified land cover class. Table 6 and 7 show the accuracy assessment results of the classification with pixel based and object oriented image analysis.

Comparing these two classification results class by class, except the land cover types “agriculture”, “orchard”, “outcrop-limestone”, “saline soils” and “Salt crust” the producer’s and user’s accuracy of the classification result using object oriented approach are higher than those using a pixel based approach (Fig. 5). This can be explained from two aspects of the two classification approaches.

From the characteristics of the two classification methods, in object oriented image analysis, object is not a single pixel takes part in the classification. Properly performed segmentation creates good image objects that facilities the extraction from the image. From the classifiers that are used in two approaches, in object oriented, the classifier is Nearest Neighbor (NN). The NN classifier has the following advantages; NN evaluates the correlation between object features favorably; NN overlaps in the feature space increase with its dimension and can be handled much easier with NN; NN allows very fast

Table 6: Accuracy of pixel-based image classification

Accuracy	Land cover types													
	Agr.	Al.	DC.	NS-S.	Or.	OC-I.	OC-L.	Pi.	Ru.	SS.	SC.	SD-L.	S-SD.	Ur
User's accuracy (%)	100	68	92	67	100	93	96	58	60	82	100	94	74	45
Producer's accuracy (%)	93	86	58	100	100	94	88	70	40	99	100	72	94	37
Overall accuracy (%)	81													

Table 7: Accuracy of object-oriented image classification

Accuracy	Land cover types													
	Agr.	Al.	DC.	NS-S.	Or.	OC-I.	OC-L.	Pi.	Ru.	SS.	SC.	SD-L.	S-SD.	Ur
User's accuracy (%)	78	100	100	88	72	100	83	80	83	98	83	100	95	91
Producer's accuracy (%)	87	94	89	95	97	93	89	91	73	94	100	89	91	89
Overall accuracy (%)	91													

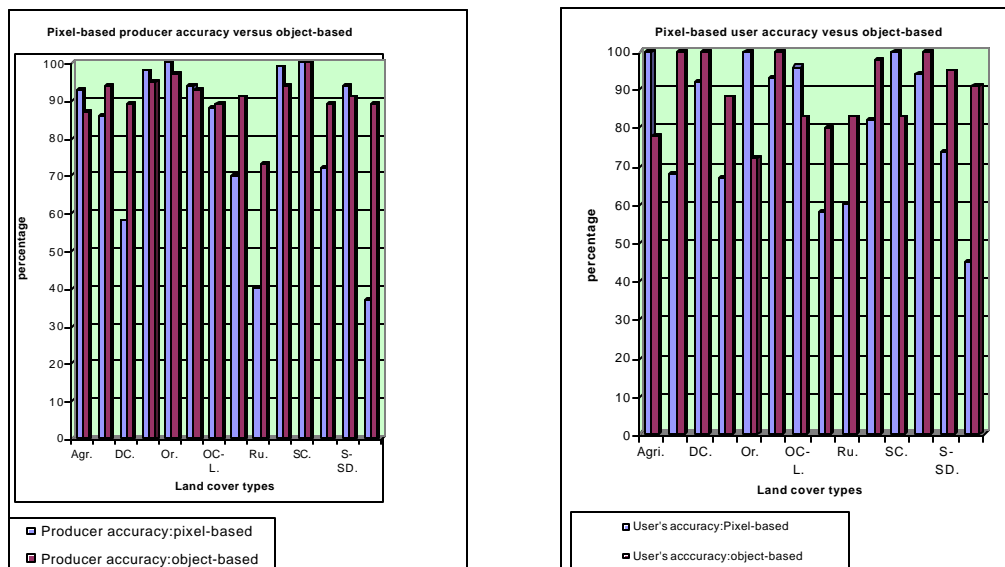


Fig. 5: Comparison between pixel-based and object-based accuracy

and easy handling of the class hierarchy for the classification.

In the pixel-based approach, the classifier is the minimum distance classifier. In this method for the spectral value of a pixel to be classified the distance towards the class means are calculated, if the shortest (Euclidian) distance to class mean is smaller than the user-defined threshold, then this class name is assigned to the output pixel, else the undefined value is assigned.

REFERENCES

1. Benz, U.C., P. Hofmann, G. Willhauck, Lingenfelder and M. Heynen, 2003. Multi-resolution, object-oriented fuzzy analysis of remote Sensing data for GIS-ready information. ISPRS Journal of Photogrammetry and Remote Sensing, 58: 239-258.

2. Dowine, A.J., D.W. Donnan and A.J. Davison, 1999. Review of Scottish natural heritages work in suitable marine biotope mapping using Remote Sensing. Intl. J. Remote Sensing, 20: 585-592.

3. eCognition User guide, 2000. <http://www.definiens-iamging.com>

4. Flanders, D., H. Mryka and P. Joan, 2003. Preliminary evaluation of eCognition object-based software for cut block delineation and feature extraction, Can. J. Remote Sensing, 20: 441-452.

5. Geneletti, D. and B.G.H. Gorte, 2003. A method for object-oriented land cover classification combining Tm data and aerial photographs. Intl. J. Remote Sensing, 24: 1237-1286.

6. Gorte, B., 1998. Probabilistic segmentation of remotely sensed images. ITC, Publication 63, Ph.D Thesis, ITC, Enchede.

7. ILWIS 3.0 User's Guide, 2001. International Institute for Aerospace survey and earth science (ITC). Enschede, The Netherlands.
8. Jensen, L.L.F. and B.G.H. Gorte, 2001. Principle of remote sensing, Chapter 12 Digital image classification, ITC, Enchede, 2nd Edn. The Netherlands.
9. Kushwaha, S.P.S., S.K. Subramanian, G.C.H. Chennaiah, J. Ramana, S.V.C. Murthy, Kameswara Rao, A. Perumal and G. Behara, 1996. Remote sensing and GIS Methods for sustainable rural development. *Intl. J. Remote Sensing*, 17: 3055-3069.
10. Lillesand, T.M., R.W. Kiefer and J.W. Chipman, 2004. Remote sensing and image interpretation. 5th Edn. John Wiley and Sons, Inc. USA.
11. Martin Baatz, B. Ursula, D. Seyed, H. Markus, H. Astrid, H. Peter, L. Iris, M. Matthias, S. Malte, W. Michaela and W. Gregor, 2004. eCognition user guide. Definiens Imaging GmbH, München Germany.
12. Price, J.C., 1994. How unique are spectral signatures. *Remote Sensing of Environment*, 49: 181-186.
13. Tso, B. and P.M. Mather, 2001. Classification methods for remotely sensed data. Taylor and Francis, Inc.

Direct and Latent Effects of Two Chitin Synthesis Inhibitors to *Spodoptera littoralis* Larvae (Boisd.)

¹S.M. Abdel Rahman, ²E.M. Hegazy and ¹A.E. Elwey

¹Central Laboratory of Pesticides, Cairo, Egypt

²Department of Economic Entomology, Faculty of Agriculture,
Alexandria University, Alexandria, Egypt

Abstract: The direct and latent effects of the growth inhibitor Lefenuron EC₅₀ [N-{2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenylaminocarbonyl}-2, 6-difluorobenzamide] and the combination of Lefenuron/Deltanet EC₂₃₅ [O-n-butyl-O-(2, 2-dimethyl-2, 3-dihydrobenzofuran-7-yl)-N-N'-dimethyl-N-N'-thiodicarbamate] on the development of *Spodoptera littoralis* larvae were tested. Different concentrations of each compound were incorporated into the meridic diet of *S. littoralis* larvae. The newly moulted 3rd larval instars were fed for 24 or 48 hours on the treated diet. Both compounds proved to be toxic to the test insect larvae. Lefenuron proved to be more toxic than Lefenuron/Deltanet. *S. littoralis* larvae suffered from more mortality when they were fed for a longer period on the treated diet. The affected larvae ceased feeding within 48 hours and most deaths occurred during moulting to the fourth instars. Incorporating 0.18 ppm of Lefenuron into the diet induced 100% mortality. No significant differences were detected in periods of larvae, prepupae or pupae of survived individuals of both compounds. However, both compounds had delayed effects on survived treated larvae. Some larvae failed to pupate successfully. In some cases, the delayed effects were manifested in the pupal or adult stages. This was expressed by pupal or adult deaths and significant reduction in the reproductive potential of apparently unaffected moths resulting from treated larvae. The effect was a concentration-dependent. Several insect parasitoids are associated with *S. littoralis* larvae. So, when insect growth inhibitors should be used for *Spodoptera* larvae control, dosages and timing of application should be carefully considered.

Key words: *Spodoptera littoralis* % insect growth inhibitors % delayed effects

INTRODUCTION

The cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera : Noctuidae) is one of the key pests that cause great damage to cotton plants as well as other field and vegetable crops in Egypt [1-3]. The widespread and continuously increasing use of different types of nonselective pesticides in cotton fields in Egypt and elsewhere disturb the biological balance and cause outbreaks of insect and mite pests.

The effect of pesticides on the non-target organisms was represented by the destruction of entomophagous agents associated with the cotton leafworm and primary or secondary pest-upsets were recorded in Egypt in areas treated extensively with insecticides [4, 5]. For instance, the rate of parasitism in the cotton leafworm, especially in

the fall generation, was about 75% during the years 1968-1972 [6], before the excessive use of pesticides, while it has now dropped to 1.9-6.2% in 1977. The American bollworm *Heliothis armigera* (Hubner) was observed as a serious pest in Egypt, although it was recorded by Willcocks and Bahgat [1] as a minor pest. The other minor pests that have risen to pest status on cotton plants in recent years are the white fly, *Bemisia tabaci*, stink bugs, *Nezara viridula* and the leafhopper, *Empoasca lylica* beside different species of tetranychid mites.

Recent reports indicate that cotton fields are the main areas where large-scale aerial and ground applications of pesticides are used. This has led to an increasing concern over both the immediate and long-term effects of such very toxic chemicals on the non-target organisms in the cotton fields [5].

The pest control strategies for the future are directed towards the wise and carefully monitored use of compounds that are non or less toxic to man, plants and all classes of existing beneficial creatures. Therefore, the integrated control concepts has been developed through the integration of biological and chemical methods. This concept was broadened to include all control methods [7]. The concept of pest management has now been extended to cover all classes of pests and it is commonly referred to as Integrated Pest Management (IPM).

In an integrated pest management programs, one should try to use selective pesticides or those which have less adverse impact on non-target organisms. In general, insect growth regulators which act as chitin synthesis inhibitors or juvenile hormone analogs have been regarded as excellent integrated control insecticides because of their specificity to target pests and their general safety to vertebrates, molluscs and plants [8, 9].

Two insect growth regulators, namely Lefenuron EC₅₀ and the combination of Lefenuron/Deltanet EC₂₃₅ (Furathiocarb) have shown promise for the control of insect pests on cotton, soya beans, vegetables, deciduous fruits, grapes and citrus. These chemicals induce their effect by inhibiting chitin deposition, which results in molting failure or rupture of the new cuticle. The effect of these chemicals on *S. littoralis* larvae comprises the object of the present study.

MATERIALS AND METHODS

Rearing of the cotton leafworm: The culture of cotton leafworm, *S. littoralis* was obtained from a laboratory colony established at Department of Entomology, Faculty of Agriculture, Alexandria University. The colony of *S. littoralis* was originated from individuals collected from field crops including cotton at Alexandria. However, feral individuals were added to the colonies twice a year to maintain genetic diversity. Larvae of *S. littoralis* were reared using the medium and methods of Hegazi *et al.* [10] at 27±1°C, 60-65% RH and a 14:10 photoperiod. Eggs of *S. littoralis* were sterilized in 1% sodium hypochloride for five minutes and rinsed with tap water for a similar period. All rearing processes of experiments were conducted in incubators maintained under 14:10 (L:D) photoperiod regiment at 25±1°C and about 60% RH. Prior to any experiment, individual larvae were examined daily and the instars were determined by counting the number of head capsules shed.

Mortality curves of the larval stage: The insect growth inhibitor Lefenuron EC₅₀ [N-{2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenylaminocarbonyl}-2,6-difluorobenzamide] and the combination of Lefenuron/Deltanet EC₂₃₅ [O-n-butyl-O-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)-N-N'-dimethyl-N-N'-thiodicarbamate] were tested. The two compounds were discovered and are being developed by CIBA-GEIGY Ltd., in Basel [11, 12].

Preliminary tests were carried out to determine the suitable series of concentrations for each test material. Concentrations-response experiments with *S. littoralis* larvae were then conducted. These materials were formulated by suspending each in distilled water and mixing with the artificial diet to obtain an end volume of 50 ml diet before the agar solidified. Methylene blue dye (1%) was added to the water as a visual guide to ensure thorough mixing of the toxicants with artificial diet. Laboratory tests proved that the dye was nontoxic at that dose. The diet was poured, while still warm, into diet cups, refrigerated and tested within 2 d. By this way, four diet concentrations of Lefenuron of 0.04, 0.06, 0.08, 0.10, 0.20 ppm were prepared for experiment 1. Five concentrations of the compound Lefenuron/Deltanet (Furathiocarb) of 0.02, 0.20, 0.40, 0.80, 2.0, 6.0 ppm were selected for experiment 2. Each diet in a cup which was a treatment, was divided into small cubical portions ca. 2 cm³ and seeded each in a small plastic Petri-dish with five *S. littoralis* larvae at the beginning of zero day of the 2nd moult. Each concentration was tested against 75-100 larvae. In this way, every treatment included 15-20 Petri-dishes. For each compound, the test larvae were left to feed for 24 or 48 h and then transferred to untreated diet until the achievement of mortality counts. A control experiment was set up in the same manner but with distilled water only. The concentration-response curves of *S. littoralis* larvae were calculated from the data of larval mortality. Expected mortality frequency was determined on the basis of observed mortality frequencies produced by each chemical alone. All results were analyzed and corrected according to Abbott's formula [13].

Effects on the development of the cotton leafworm: The effects of two compounds on the developmental stages of the insect were tested. For each compound four concentrations of 0.04, 0.08, 0.12, 0.18 ppm were used. Each concentration was tested against 35-40 newly moulted 3rd instar larvae. The same procedure used in both experiments 1 and 2 was followed in this test. After

treatments, *S. littoralis* larvae were reared individually in disposable small plastic Petri dishes (3.5x1.4 cm) to obtain daily detailed records of the effect of the test duration of the last four larval instars, prepupal and pupal stages and longevity of the emerging adults.

The morphogenetically, unaffected externally, normal adults obtained in different experimental variants of the last experiment were grouped in pairs. This was achieved by pairing and confining a female resulting from treated larvae with two males from normal laboratory culture, with the intention of obtaining daily records of the effect of the test compounds on the oviposition period and egg-laying capacity of the survived adults. The caged adults were sexed in the pupal stage and kept as isolates in plastic cups (7x5.8 cm) covered with muslin. After emergence, the adults were fed daily on 10% sugar solution contained in suspended cotton plugs and provided with a piece of paper as an oviposition site. Daily records on the oviposition period and egg-laying capacity (wt. in mg) and other biological records were carefully collected.

Statistical analysis: Analysis of variance was carried out to determine if there were significant differences among results of different treatments. In case of dealing with results of two treatments, the existence of a significant difference was determined by t-test. The L.S.D method was used for comparison between the means in certain results [14].

RESULTS AND DISCUSSION

Mortality curves of the larval stage: Figure 1 shows the concentration-mortality curves after feeding newly moulted 3rd instar of *S. littoralis* larvae for 24 or 48 h on diet treated with Lefenuron. The larvae that were fed for 24 h on diets with one concentration of 0.04, 0.06, 0.08, 0.10 or 0.20 ppm produced ca. 16, 54, 65, 80 and 99% mortality, respectively. On testing other group of larvae for longer period (48 h), the same concentrations gave mortality ranged from 24 to 99%. In both, the percentage mortality of the larvae that were fed on IGR-free diet did not exceed 6.0%.

Following the bioassay statistical analysis by Litchfield and Wilcoxon [15], when the Lc-p-lines of these results were plotted (Fig. 1), they proved to be a good fit as the differences between the experimental and tabulated Chi² values were always insignificant. The slope function of the resulted curve for the larvae fed for 24 h on the treated diet with the IGR was 1.58, while it decreased to 1.36 when the larvae were allowed to feed on the treated

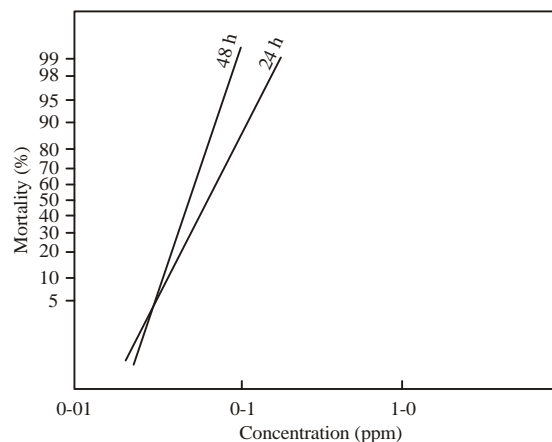


Fig. 1: Concentration-mortality curves after feeding *S. littoralis* 3rd-instar larvae for 24 or 48 h on the semi-meridic diet treated with Lefenuron

diet for 48 h. This further indicates that the cotton leafworm larvae suffered more mortality when they were fed for a longer period on the treated diet. The LC₅₀ value also reached 0.06 ppm with fiducial limits from 0.04 to 0.08 for the larvae treated for 24 h. However, a slight decrease in the LC₅₀ value (0.05 ppm) and a narrower range for the fiducial limits (0.04 to 0.05) were observed when the larvae were fed for 48 h on treated diets.

The mortality in the larval stage was clearly due to the moulting-disturbing effect of the Lefenuron. The *S. littoralis* larvae ceased feeding within 48 h and most deaths occurred while the larvae were moulting, usually between the third and fourth instar. Generally, the *S. littoralis* larvae died within the old cuticle and the newly formed cuticle was extremely thin.

Figure 2 shows the effect of exposing newly moulted 3rd instar larvae to other emulsifiable concentrates of the IGR Lefenuron/Deltanet EC₂₃₅. The larvae that were fed for 24 h on concentrations of 0.2, 0.4, 0.8, 2.0 and 0.6 ppm induced ca. 3, 11, 22, 49 and 91% mortality, respectively, while death value for the larvae fed on normal diet (control) was 6.67%. The LC₅₀ value was 1.7 ppm with fiducial limits of 1.41-2.04 ppm, while the slope value of the line was 2.59. The effect was nearly similar when *S. littoralis* larvae were left to feed on the same concentrations for 48 h. The percentage mortalities obtained were ca. 8, 16, 35, 63 and 100%, respectively. The LC₅₀ value was 1.3 ppm with fiducial limits of 1.01 to 1.66 ppm, while the slope value of the line was 3.56. At the low concentrations of 0.2 and 0.4 ppm and the intermediate ones of 0.4 and 0.8 ppm, there were no clear moulting-disturbing effects from the Lefenuron/Deltanet as most of

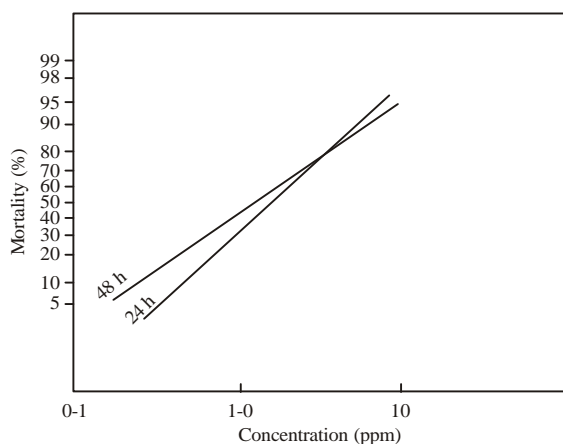


Fig. 2: Concentration-mortality curves after feeding *S. littoralis* 3rd-instar larvae for 24 or 48 h on the semi-meridic diet treated with Lefenuron/Deltanet

the tested larvae survived after treatments. However, the highest concentration (6.0 ppm) induced serious effects within the first two days after treatments in both feeding periods and many larvae suffered from moulting failure. Although the range of concentrations tested for Lefenuron were lower than those used for Lefenuron/Deltanet, the former IGR was more effective than the other. Whereas, *S. litura*, the oriental leafworm, which is a pest of cotton and tobacco in Asia and Australia, was susceptible to diflubenzuron [16, 17]. *S. exigua*, the beet armyworm, was not susceptible to

diflubenzuron, but penfluron and chlorfluazuron were very effective. This species is able to metabolize or detoxify or eliminate diflubenzuron and escape its insecticidal effects [18]. It seems that *S. littoralis* larvae were able to escape the insecticidal effects of the formulation Lefenuron/Deltanet if it is used at the same concentration of Lefenuron alone.

The present work showed that the mortality was clearly caused by moulting failure of *S. littoralis* larvae. This effect is mainly induced by inhibiting chitin formation [19, 20], thereby causing abnormal endocuticular deposition and abortive moulting [21]. Other effects of the chitin inhibitor-compounds were reported. They are known to act on the peritrophic membrane by affecting its chitin-protein structure, hindering its role in protecting the secreting cells from any damage [22].

Development of survived treated *S. littoralis* larvae: The results of the effect of Lefenuron and Lefenuron/Deltanet on the development of survived treated *S. littoralis* larvae are shown in Table 1. For Lefenuron-treated larvae, all larvae treated with 0.18 ppm died during moulting from third to fourth instar. However, some of larvae treated with lower concentrations survived treatments. The duration of the last four larval instars in the control ranged from 8 to 11 days with an average of 9.18 ± 0.15 days. This range lasted for 8 and 10 days in treated larvae with an average of 8.34 ± 0.01 , 8.52 ± 0.51 and 9.0 ± 0.0 days for those fed on diets containing 0.04, 0.08

Table 1: Duration (in days) of survived *S. littoralis* stages (mean±SE) after treatment of newly 3rd-instar larvae by Lefenuron and Lefenuron/Deltanet

Con. (ppm)	Duration (in days)		Pupae & Adult longevity (in days)			
	Larvae	Pre-pupae	%	&	%	&
Lefenuron						
0.00	9.18a±0.15	2.85a±0.10	8.50a±0.15	9.44a±0.16	7.45a±0.21	5.56a±0.18
0.04	8.34a±0.10	2.52a±0.10	8.33a±0.15	5.58a±0.15	7.33a±0.19	5.50a±0.21
0.08	8.52a±0.51	2.42a±0.51	8.38a±0.74	8.38a±0.52	7.50a±0.55	5.14a±0.69
0.12	9.00a±0.00	2.50a±0.52	7.80a±0.84	8.60a±0.53	6.30a±0.96	3.80b±0.50
0.18	All larvae died					
Lefenuron/Deltanet						
0.00	10.05a±0.08	2.68a±0.08	8.50a±0.12	9.10a±0.16	7.94a±0.22	5.90a±0.16
0.04	10.29a±0.08	2.50a±0.09	8.30a±0.12	9.00a±0.16	7.53a±0.15	5.77a±0.20
0.08	10.09a±0.11	2.88a±0.12	8.31a±0.11	9.08a±0.15	6.88a±0.27	5.75a±0.18
0.12	9.65a±0.14	2.80a±0.13	7.35a±0.12	7.55a±0.16	6.41a±0.27	5.09a±0.21
0.18	9.47a±0.11	2.50a±0.10	7.31a±0.13	7.87a±0.19	6.46a±0.33	5.20a±0.20

For each set of *S. littoralis* stage, figures marked by the same letter are not significantly different ($p > 0.05$)

and 0.12 ppm, respectively. However, no significant differences were found between the larval periods in all treatments.

On reaching the prepupal stage, some larvae suffered from partial moult inhibition and died during their attempt to shed the old cuticle. The prepupal duration in the control ranged from 2 to 4 days with an average of 2.85 ± 0.10 days, while those of surviving prepupae of treated larvae varied from 2 to 3 days in all treatments. The analysis of variance proved no significant differences between the durations of the prepupal period of all the tested larvae. On reaching the pupal stage, some of the pupae resulting from the treated larvae were apparently morphologically perfect individuals. The duration of these pupae ranged from 7 to 10 days for males and 8 to 9 days for females. There were no significant differences between the pupal period of all resulting pupae including those of the control. However, delayed effects were observed among some of treated larvae. Some of the latters developed into malformed pupae and some failed to pupate successfully instead they formed larval pupal intermediate. All these larvae died while attempting to moult. The adults with eclosion problems were, by far, one of the most serious effects that faced some adults resulting from the treatments with intermediate concentrations (0.08 and 0.12 ppm). This phenomenon was noted when the affected adults attempted to extricate themselves from the pupal skin. In other cases, some adults freed the abdomen successfully from their pupal exuvia, but the thorax and head remained bound to the pupal skin and others having vestigial wings.

The effect of Lefenuron/Deltanet on the development of *S. littoralis* larvae is shown in Table 1. Of the treated larvae 77.78% survived the highest tested concentration of 0.18 ppm and reached their adult stage. The affected larvae died as larval-pupal, pupae, pupal-adult intermediates or dead adults inside their pupal skin or emerged as imperfect adults. Most of these symptoms are similar to the Lefenuron treatments.

The development of apparently non-affected larvae with higher concentrations (0.12 and 0.18 ppm) was one day faster in the duration of the larval or pupal stages than those larvae which survived lower concentration or ones that were fed on Lefenuron/Deltanet diet-free. However, the differences between either of larval, prepupal or pupal stages of the surviving larvae and the controls was insignificant.

Figure 3 shows percentages of *S. littoralis* moths resulting from larvae treated with Lefenuron and Lefenuron/Deltanet. The effect of Lefenuron treatments

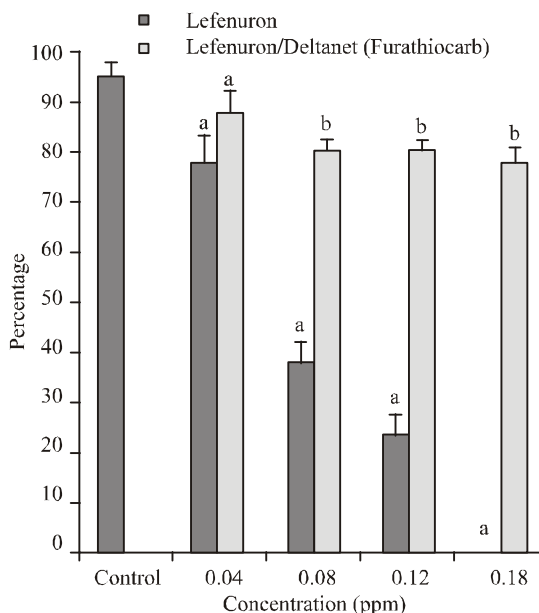


Fig. 3: Percentage of *S. littoralis* moths (mean±SE) resulting from larvae treated with different concentrations of IGR's. Mean values followed by different letter above each bar within each set are significantly different at p=0.05

were dose-dependent. The percentage of the formed adults was 92.50% when the larvae were fed on Lefenuron dite-free. This figure dropped significantly with increasing the concentration of the compound in the diet. The doses of 0.04, 0.08, 0.12 and 0.18 ppm provided 77.78, 37.80, 23.33 and 0.0% adults, respectively. However, the same doses of Lefenuron/Deltanet were sublethal and did not adversely affect larval or adult survival. There was a significant effect on the percentage of the adults resulting from the larvae fed on diets containing 0.08, 0.12 or 0.18 ppm, when compared with either those produced from the control larvae or those treated with the lowest concentration (0.04 ppm). These data further indicate that Lefenuron/Deltanet at concentrations not lethal to the treated 3rd-instar larvae or the pupae caused changes in emerging adults (Fig. 3). These results of Lefenuron/Deltanet were similar to those reported by Madore *et al.* [23] on the molt-inhibiting IGR, uc-62644 when the 6th - instar larvae of *Choristoneura funiferana* (Clemens) were treated with sublethal concentrations of the compound. Madore *et al.* [23] also reported that where chemicals such as uc-62644 are used as larvicides, workers should be aware that delayed effects may occur in later stages of the survivors, a case which can be applied for the Lefenuron/Deltanet too.

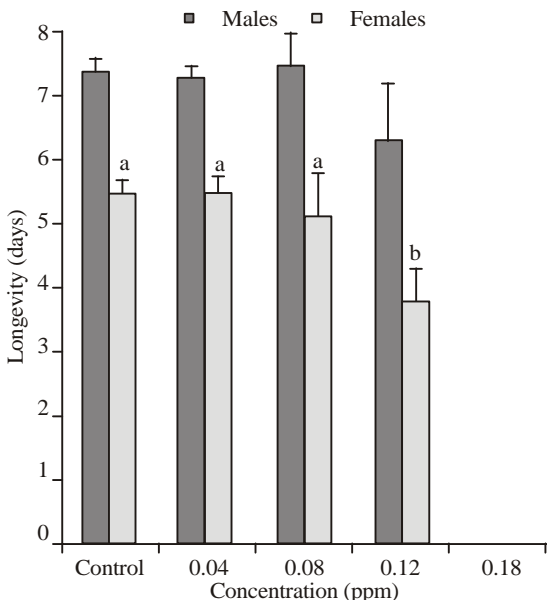


Fig. 4: Longevity (in days) of *S. littoralis* moths (mean±SE) resulting from 3rd instar larvae treated with Lefenuron. Mean values followed by different letter above each bar among female moths are significantly different at p=0.05

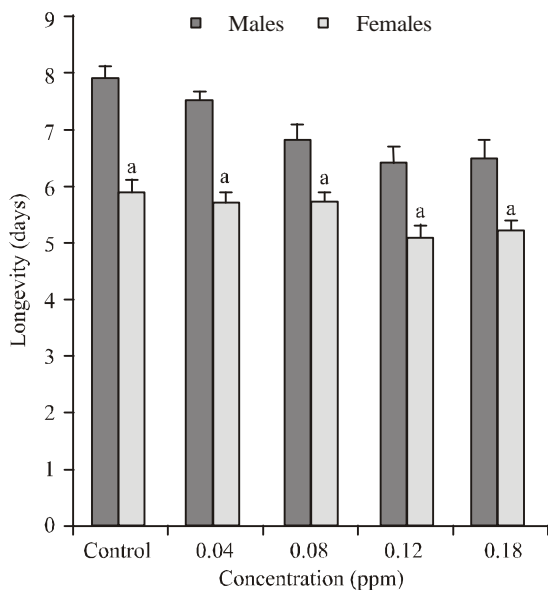


Fig. 5: Longevity (in days) of *S. littoralis* moths (mean±SE) resulting from 3rd instar larvae treated with Lefenuron/Deltanet (Furathiocarb). Mean values followed by different letter above each bar among female moths are significantly different at p=0.05

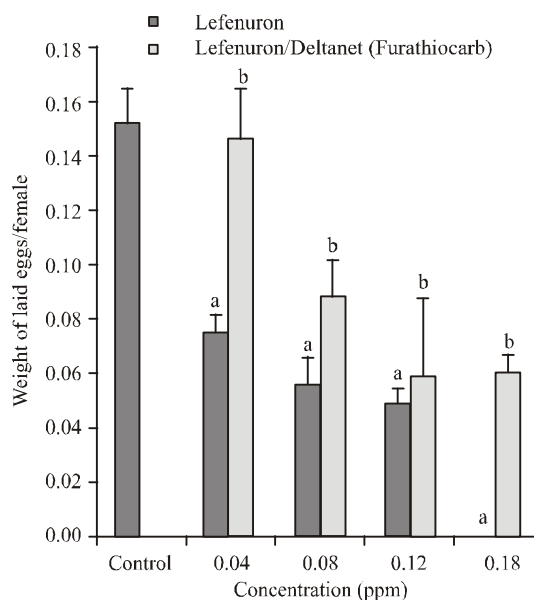


Fig. 6: Egg laying capacity (in mg) of *S. littoralis* moths (mean±SE) resulting from larvae treated with different concentrations of IGR's. Mean values followed by different letter above each bar within each concentration set are significantly different at p=0.05

Other delayed effects of the tested compounds can be seen in the longevity of apparently perfect adults that resulted from treated 3rd-instar *S. littoralis* larvae (Fig. 4 and 5). The first two doses (0.04 and 0.08 ppm) of Lefenuron incorporated into the test diets did not adversely affect the longevity of adults derived from treated larvae. The resulting adults in the control lived for an average of 7.45 and 5.56 days for male and females, respectively. These figures were not significantly greater than those recorded in the treatments (Table 1). However, the longevity of females produced by larvae fed on 0.12 ppm of the same compound incorporated into the diet decreased significantly to an average of 3.8 days (Fig. 4). On the contrary, the adults developed from larvae fed on the tested doses of Lefenuron/Deltanet lived almost as long as control adults (Fig. 5 and Table 1).

Egg-laying capacity of survivor female moths: The adult fecundity of externally normal female moths of *S. littoralis* resulting from treated larvae is shown in Fig. 6. The effect was concentration-dependent reduction in the reproductive potential of the emerging adults. The average weight of laid eggs/control female reached

0.152 mg. This figure was significantly reduced for female resulting from Lefenuron-treated larvae (Fig. 6).

In case of *S. littoralis* females formed from Lefenuron/Deltanet treated larvae, the lower concentration of 0.04 ppm did not adversely affect the egg-laying capacity of the female moths. However, by increasing the incorporated concentration of the same compound into the larval diet, the weight of the deposited eggs by the surviving adults significantly decreased and was concentration-dependent.

The adult fecundity of *S. littoralis* was disrupted by both Lefenuron or Lefenuron/Deltanet treatments. Surviving females from treated larvae oviposited significantly fewer eggs. Madore *et al.* [23], working on the spruce budworm, *C. fumiferana* demonstrated similar results. The experimental insect growth regulator uc-62644 fed at sublethal concentration to 6th-instar larvae caused a dose-dependent reduction in reproductive potential of the emerging adults.

Finally, as with all IGR's [24], the present tested compounds have a prolonged knockdown time on the target insect (*S. littoralis* larvae). However, results of the present work indicated that many *S. littoralis* larvae cease feeding within 48 h after treatment. The prolonged delayed effects should not be a limiting consideration for including these IGR's in management programs. Nevertheless, the effects of these compounds on the non-target insects are some of important concepts which should be investigated in detail.

REFERENCES

1. Willcocks, F.C. and S. Bahgat, 1937. The insect and related pests of Egypt. Vol. 1, Part 2, Insect and mites injurious to the cotton plant. Royal Agric. Soc. Entomological Section, Cairo, Egypt.
2. Bishara, I., 1954. The cotton worm, *Prodenia litura* F., in Egypt. Soc. Ent. Egypt. Bull., 18: 288-420.
3. Moussa, M.A., M.A. Zaher and F. Kotby, 1960. Abundance of the cotton leafworm *Prodenia litura* (F.), their effect on biology. Bull. Soc. Ent. Egypt, 44: 241-251.
4. Kamal, M., 1951. The biological control of the cotton leafworm *Spodoptera littoralis* F. in Egypt. Bull. Soc. Ent. Egypt, 35: 221-270.
5. Hafez, M., 1972. Certain factors influencing mortality of natural enemies of cotton pests. Agric. Res. Rev., Cairo, 50: 1-16.
6. Attia, H.H., 1977. Ecological assessment of pesticide management on terrestrial ecosystem in Egypt. Proceedings of the VC. AID University of Alexandria, A.R.E. Seminar Workshop in Pesticide Management, March 5-10.
7. Smith, R.F., 1970. Pesticides. Their use and limitation in management. (in concept of pest management. Robb, R.L. and F.E. Guthrie, (Eds.). N.C. State University, Raleigh).
8. Wilkinson, J.D., K.D. Bieveri, C.M. Ignoffo, W.J. Pons, R.K. Morrison and R.S. Seay, 1978. Evaluation of diflubenzuron formulations. J. Ga. Entomol. Soc., 13: 227-236.
9. Deakle, J.P. and J.R. Bradly, 1982. Effects of early season applications of diflubenzuron and azinphosmethyl on population levels of certain arthropods in cotton fields. J. Ga. Entomol. Soc., 17: 200-204.
10. Hegazi, E.M., A.M. El-Minshawy and S.M. Hamed, 1977. Suitability of *Spodoptera littoralis* larvae for development of *Microplitis rufiventris*. J. Agric. Sci. Camb., 89: 659-662.
11. Anonymous, 1988. Deltanet 235EC insecticide against foliar and soil pests. Ciba-Geigy Limited, Basle, Switzerland, Agricultural Division, C: 11-4002.
12. Anonymous, 1989. CGA 184699 insect growth inhibitor for cotton, soya, vegetables, potatoes, deciduous fruits, grapes and citrus. Ciba-Geigy Limited, Basle, Switzerland.
13. Abbotts, W.S., 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
14. Bishr, M.A. and M.M. El-Robi, 1976. Introduction on statistical methods and experiments design, in Arabic. Textbook, Dar El-Maarif, Alexandria.
15. Litchfield, J.I. and F. Wilcoxon, 1949. Staniford research laboratories. Entomologie, 89: 193-198.
16. Sundaramurthy, V.T. and M. Balasubramiam, 1978. Effect of an inhibitor of chitin deposition in tobacco caterpillar (*Spodoptera litura* Fb.) under induced hyper hormone condition. Z. Ang. Ent., 85: 317-321.
17. Santharam, G. and M. Balasubramianiam, 1980. Note on the control of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) on tobacco with a nuclear polyhedrosis virus and diflubenzuron. Indian J. Agric. Sci., 50: 726-727.
18. Granett, J., B. Bisabri-Ershadi and M.J. Hegazi, 1983. Some parameters of benzoylphenyl urea toxicity to beet armyworm. J. Econ. Entomol., 13: 133.

19. Ishaaya, I. and J.E. Casida, 1974. Dietary TH 6040 alters composition and enzyme activity of house fly larval cuticle. *Pestic. Biochem. Physiol.*, 4: 484-490.
20. Post, L.C., B.J. de Jong and W.R. Vincent, 1974. 1-(2,6-disubstituted benzoyl)-3-phenylurea insecticides: Inhibitors of chitin synthesis. *Pestic. Biochem. Physiol.*, 4: 473-483.
21. Mulder, R. and M.J. Gijswijt, 1973. The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition. *Pestic. Sci.*, 4: 737-745.
22. Clark, L., G.H.R. Temple and J.F.V. Vincent, 1977. The effects of chitin inhibitor-Dimilin on the production of peritrophic membrane in the locust, *Locusta migratoria*. *J. Insect. Physiol.*, 23: 241-246.
23. Madore, D.C., B.G. Drion and B.J. Dimond, 1983. Reduction of reproductive potential in spruce budworm (Lepidoptera: Tortricidae) by a chitin inhibiting insect growth regulator. *J. Econ. Entomol.*, 76: 708-710.
24. Chandler, L.D., S.D. Pair and W.E. Horison, 1992. RH 5992, a new insect growth regulator active against corn earworm and fall armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.*, 85: 1099-1103.

INSTRUCTIONS TO AUTHOR

AIMS AND SCOPE

American-Eurasian Journal of Agricultural & Environmental Sciences (AEJAES) publishes original scientific work related to strategic and applied studies in all aspects of agricultural science, as well as reviews of scientific topics of current agricultural relevance. The papers, which are of international interest but feature a northern perspective, cover a wide range of topics in basic and applied research. The topics are agricultural economics, agricultural engineering, animal science, environmental science, food science, nutrition science, horticulture, and plant and soil science. Submissions are internationally refereed.

HOW TO SUBMIT

American-Eurasian Journal of Agricultural & Environmental Sciences welcomes contributions in the form of original research papers, review articles, technical reports and short communications in all aspects of Agriculture & Environmental Sciences. When a manuscript is submitted, it is assumed that the work has not been published elsewhere and that it is not simultaneously being submitted to another journal. If a manuscript is accepted, the authors will be requested to assign the copyright of the article to American-Eurasian Journal of Agricultural & Environmental Sciences.

ELECTRONIC SUBMISSIONS ARE ENCOURAGED

It is recommended to submit manuscript (MS Word file) through e-mail (idosi@idosi.org or idosi.editor@gmail.com) as an attachment for quick evaluation.

To facilitate editorial work, please keep the editorial office informed of any changes in your address, e-mail address and telephone number.

STYLE OF MANUSCRIPTS

Manuscripts should be written in clear, concise and grammatically correct English. Indent new paragraphs. The manuscript should be presented in the following order.

Headings: Headings should be upper case and in bold type. Sub-headings should be lower case and in italics.

Figures: Figures should be high-quality. A figure legend should be supplied. Color figures can only be accepted if the cost of reproduction is borne by the authors.

Tables: Number in order of mention in the text and Table should be in text form not show as an image.

References: Bibliographic references in the text appear like [1, 2, 5, 6], using square brace. References should be numbered consecutively in text.

REFERENCES

Journal Articles: Ouyang, D., J. Bartholic and J. Selegan, 2005. Assessing Sediment Loading from Agricultural Croplands in the Great Lakes Basin. *Journal of American Science*, 1(2): 14-21.

A Book: Durbin, R., S.R. Eddy, A. Krogh and G. Mitchison, 1999. *Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids*. Cambridge University Press.

A Chapter in a Book: Leach, J., 1993. Impacts of the zebra mussel (*Dreissena polymorpha*) on water quality and fish spawning reefs of Western Lake Erie. In *Zebra mussels: biology, impacts and control*, Eds., Nalepa, T. and D. Schloesser. Ann Arbor, MI: Lewis Publishers, pp: 381-397.

A Report: Makarewicz, J.C., T. Lewis and P. Bertram, 1995. *Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992*. U.S. EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.

Conference Proceedings: Stock, A., 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.

A Thesis: Strunk, J.L., 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, M. S. thesis, Michigan State Univ., East Lansing, MI.

ABBREVIATIONS, UNITS ETC.

Authors should follow internationally agreed rules especially those adopted by the IUPAC-IUB Commission on Biochemical Nomenclature (CBN). The journal will essentially follow the rules defined in the IUPAC Manual of symbols and terminology for physico-chemical quantities and units (Butterworths, London), 1970.

COPYRIGHT AND PERMISSIONS

By submitting a manuscript to the editor or publisher you are deemed to have granted permission to publish the manuscript.

PROOFS

Proofs will be sent via e-mail as an Acrobat PDF (Portable Document Format) file. Acrobat Reader will be required in order to read the PDF. This software can be downloaded from the following website:

<http://www.adobe.com/products/acrobat/readstep2.html>

This will enable the file to be opened, read on screen and printed out in order for any corrections to be added.

PRINTING / PROCESSING / DELIVERY COST

For single article up to to 6 final pages will be US 95 \$. After that IDOSI will charge extra for each single page.

The publishing contents for author contains:
1 Journal Copy + 05 off prints + Online

AUTHORSHIP

For papers to be published in American-Eurasian Journal of Agricultural & Environmental Sciences each author should have participated sufficiently in the work to take public responsibility for the content. (This statement is taken from the authorship policy adopted by the International Committee of Medical Journal Editors and published in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, 1994 and N Engl J Med 336: 309-315,1997.)

CORRECTIONS TO PUBLISHED ARTICLES

If necessary, corrections of significant errors in published articles will be published in a later issue of the Journal. Within two months after publication, authors are requested to bring any errors to the attention of the managing editor.

METHODS OF PAYMENT

Procedure 1: You can send money through Western Union with receiver name **Muhammad Zeeshan**. (After send please inform through E-mail about Money Transfer Control Number (MTCN))

Procedure 2: IDOSI also receive Printing / Processing / Delivery charges through Bank Draft in favor of **IDOSI Publications** and send it to our postal address :

IDOSI Publications
P-100, St. # 7, Sohailabad,
Peoples Colony # 2, Faisalabad, Pakistan

Procedure 3: Our Bank Route for F.T.T Payment into Our Account.

US \$ Route:

Receiver's Correspondent:

SWIFT: MRMDUS33
HSBC Bank, NY, USA

Account with Inst-Name & Address:

SWIFT: MEZNPKKA
Meezan Bank Ltd., Karachi, Pakistan

Beneficiary Customer-Name & Address:

Account No: 0401-020-0000-2227
IDOSI Publications
Serena Hotel Branch,
Branch Code: 0401
Faisalabad, Pakistan