American-Eurasian J. Agric. & Environ. Sci., 2 (1): 01-05, 2007
ISSN 1818-6769

Effects of De-Awning and Moisture Content on Husking Characteristics of Paddy in Rubber-Roll Husker

Saeid Minaei, Mohammad Reza Alizadeh, Mohammad Hadi Khoshtaghaza and Timor Tavakoli

Department of Agricultural Machinery Engineering, Tarbiat Modares University, Tehran, IR Iran

Abstract: The effects of de-awning on husking characteristics of paddy varieties (Hashemi and Binam) in a rubber-roll husker were avaluated as function of grain moisture content. In the case of Hashemi variety, the husking ratio significantly decreased from 81.61 to 76.45% and 85.19 to 78.22%, Husking index from 73.88 to 67.71% and 76.59 to 67.93% ; husking time increased from 63.20 to 74.40 and 38.80 to 46.40 sec, respectively for awned and de-awned paddy as moisture content increased from 9.06 to 14.92% w.b. For Binam variety, the husking ratio varied from 80.59 to 71.69% and 83.04 to 73.73%, the husking index from 71.40 to 62.43% and 72.27 to 62.35%; the husking time increased from 54.70 to 63.90 sec and 32.00 to 38.40 sec, respectively for awned and de-awned paddy with increasing moisture content from 8.92 to 14.78% w.b. It is concluded that de-awning has a significant positive effect on husking quality as well as husking time.

Key words: Paddy de-awning % husking characteristics % husking index

INTRODUCTION

Rice (*Oriza sativa* L.) is an important staple food in Iran. It is grown on an area of about 615 thousand ha with a total paddy production of about 3.0 million ton. Main areas of rice cultivation in Iran are located in Mazandaran and Guilan provinces producing 75 percent of Iran's rice crop. Both high yielding and local varieties are grown in the rice cultivated areas in the country. In Guilan province however, the most popular varieties grown are local and aromatic varieties such as Hashemi and Binam. These varieties are characterized by long kernels having awns. The presence of awn influences the physical and morphological characteristics of these types of rice varieties, that causes difficulty in flow through chutes and hopper orifices [1].

Milling is the final step in rice post-harvest processing. It includes pre-cleaning, husking, whitening and grading [2]. In the rice milling process, paddy is first thoroughly cleaned by a paddy cleaner and then the husk is removed by any of the existing huskers. Rubber-roll husker is the most popular machine for husking of paddy in milling operation because of its better performance in quality and quantity in comparison to other husker machines. The performance of a husker is not only governed by the working parameters of the machine (engineering factors) but also the physical and morphological characteristics (varietal properties) of the paddy to be husked [3]. The grains subjected to the husking process must be suitably prepared first so that their physical properties meet the requirements of the process. A recent study showed that the de-awning of paddy influenced such physical properties as the thousand-grain mass, bulk and true densities, angle of repose and static coefficient of friction [1].

Several investigators have already identified many factors affecting husking quality of paddy in rubber-roll husker. Firozi and Alizadeh [4] studied the effects of moisture content and the peripheral speed difference of rolls on husking quality for Binam and Khazar rice varieties [4]. The highest husking ratio was obtained at 8% moisture content compared to 12 and 14% moisture content levels (% w.b.). Payman *et al*. [5] reported that grain moisture content and clearance between husker rolls significantly affected husking index of rice varieties.

Although many factors affecting husking characteristics of paddy in rubber roll huskers have already been studied, however there is no information on the effect of de-awning on husking quality of paddy. There is a general awareness in the milling industry that paddy with awns causes problems contributing to handling and processing, particularly in the husking

Corresponding Author: Dr. Mohammad Reza Alizadeh, Department of Agricultural Machinery Engineering, Tarbiat Modares University, Tehran, IR Iran

stage, but no quantitative data exist about their effects on husking parameters. The objective of this study was to investigate the effects of de-awning and moisture content of paddy on husking ratio, hausking time and the husking index in rubber-roll husker.

MATERIALS AND METHODS

This study was carried out at the Agricultural Engineering Division, Rice Research Institute, Rasht, Iran. Two varieties of rice having awns namely Hashemi and Binam were selected from popular rice varieties in Iran. Initial moisture content of the grains was determined by oven drying at 103 for 48 h [6] and was 14.78 and 14.92% w.b. for Binam and Hashemi varieties, respectively. The experiment was conducted at moisture content range of 8.92 to 14.92% w.b. To obtain the desired moisture level below the initial moisturte contents, paddy was kept in an oven at a constant temperature of 43°C until the desired mass of the samples were obtaind [7]. De-awned samples of paddy were provided by rubbing the awned paddy between fingers and percentage of de-awning was calculated on the basis of weight of de-awned paddy.

In order to study the effects of de-awning and moisture content on husking quality of the two varieties, 150g awned and de-awned paddy at each moisture content level was husked using the laboratory rubber rolls husker (Satake Co. Ltd., Japan). After husking, the unhusked paddy was handpicked and separated from the brown rice and weight of each part was measured. The percentage of head brown rice was determined by hand-sorting of broken kernels. A kernel having equal to or more than 75% intact tissue was considered as whole kernel [8]. The husking ratio of awned and de-awned paddy at different moisture contents was determined using the following relation [9]:

$$H_r = 100(1 - W_2 / W_1) \quad (1)$$

Where;
H_r = Husking ratio%
W_1 = Mass of sample before husking, g
W_2 = Mass of unhusked paddy in the final product, g

To determine the husking time of the samples, the time needed to dehusk paddy was measured using a timer and the average of three replications was recorded. The clearance between the rubber rolls and the peripheral speed difference were adjusted according to findings of a previous study [5]. These were kept consatat during all experiments. The husking index of paddy in rubber-roll husker was calculated using the following equation [9]:

$$H_i = 100(1 - W_2 / W_1)(W_3 / W_1 - W_2 - W_4) \quad (2)$$

Where;
H_r = Husking index, %
W_1 = Mass of sample before husking, g
W_2 = Mass of unhusked paddy in the final product, g
W_3 = Mass of brown rice in the final product, g
W_4 = Mass of husks in the final product, g

The obtained data was statistically analayzed using a Randomized Complete Block Design (RCBD) of 2×2×4 factorial experiment with three replications in each treatment and the means were compared using Duncan's Multiple Range Test (DMRT) at the 5% level.

RESULTS AND DISCUSSION

Husking ratio: Results of the effects of de-awning and moisture content on husking ratio of paddy are presented in Fig. 1. Data revealed that the husking ratio of awned and de-awned paddy in Hashemi variety decreased from 81.61 to 76.45% and 85.19 to 78.22%, respectively as moisture content increased from 9.06 to 14.92% w.b. In the case of Binam, the husking ratio varied from 80.59 to 71.69% and 83.04 to 73.73%, respectively as the moisture content increased from 8.92 to 14.78% w.b. There was high significant ($p<0.01$) effect of de-awning and moisture content on husking ratio. The analysis of variance further confirms the observations regarding the effect of main variables on the husking ratio. The effect of variety (A), de-awning (B) and moisture content (C) significantly affected the husking ratio at 1% level. However, the first and second order interactions between the variables were not statistically significant (Table 1).

The husking ratio of grains in a rubber-roll husker depends on the physical properties of the paddy to be husked and the operational conditions of the husker such as the clearance between rolls and the peripheral speed difference of the fast and slow rolls. Since the working parameters of the machine were considered constant during the experiments, then it can be said that the variations in husking ratio are related to de-awning and moisture content of the paddy. In all the varieties tested, the highest husking ratio was obtained at the 9% moisture content level and the lowest at 15% as shown in Fig. 1. This behaviour of decrease in husking ratio with increasing moisture content is probably due to the fact

Table 1: Analysis of variance for husking characteristics of awned and de-awned paddy at different moisture contents

Source of Variation (SV)	DF	Mean Square (MS)		
		Husking ratio	Husking time	Husking index
Replication	2	0.0332^{ns}	2.4300^{ns}	1.2280^{ns}
Variety (A)	1	0.3040**	855.1400**	72.6400**
Type of paddy (B)	1	0.1770**	6907.2000**	1.0290^{ns}
Moisure content (C)	3	0.4016**	168.8000**	59.8980**
A×B	1	0.0006^{ns}	23.8000^{ns}	0.8390^{ns}
A×C	3	0.0173^{ns}	1.9600^{ns}	0.8720^{ns}
B×C	3	0.0029^{ns}	12.1700^{ns}	1.0910^{ns}
A×B×C	3	0.0024^{ns}	0.7500^{ns}	0.3200^{ns}
Error	30	6.9000	0.0060	0.7310

**Significant at 1% level, * Significant at 5% level, ns = non significant, DF= degrees of freedom

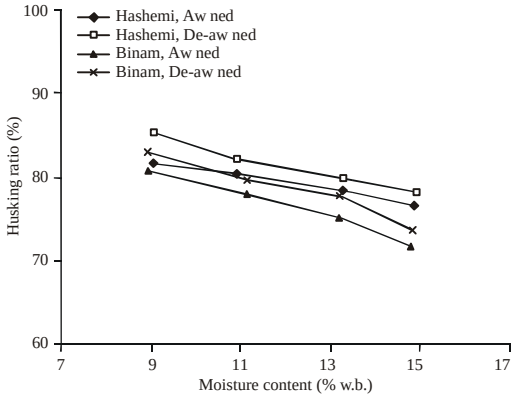

Fig. 1: Effect of de-awning and moisture content on husking ratio of paddy in rubber-roll husker for Hashemi and Binam varieties

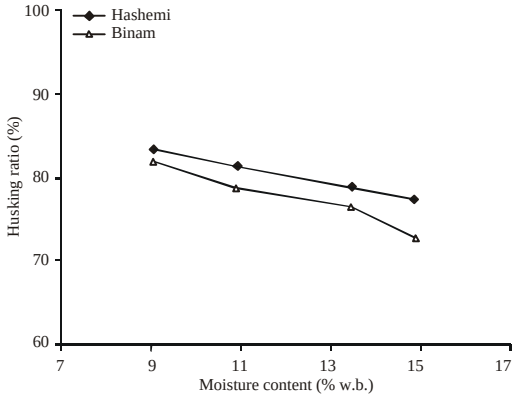

Fig. 2: Effect of moisture content on husking ratio for Hashemi and Binam varieties

that at high moisture level the husk is more firmly attached to the kernel and does not easily split [10]. Similar treneds were reported by other researchers for different rice varieties [4, 5]. It can be seen (Fig. 2) that at each moisture content level, the husking ratio of Hashemi was higher than that of Binam variety. This may be attributed to the Hashemi grains being longer than Binam, resulting in a higher contact area for Hashemi compared to Binam.

Husking time: The analysis of variance showed that variety (A), de-awning (B) and moisture content (C) significantly affected hulling time of the paddy samples in the rubber-roll husker at 1% level (Table 1). However, interaction effects of the independent variables were not significant. It was observed that for each type of paddy (awned and de-awned), husking time of the samples increased with increasing moisture content. The husking time of the awned and de-awned paddy of Hashemi grains increased from 63.20 to 74.40s and 38.80 to 46.40s, respectively as moisture content increased from 9.06 to 14.92% w.b. In the case of Binam variety, it increased from 54.70 to 63.90 and 32.00 to 38.40 sec, respectively as the moisture content increased from 8.92 to 14.78% w.b. At each moisture content level studied, the hulling time of awned paddy was significantly ($p<0.01$) higher than that of de-awned paddy (Fig. 3). This is due to the increased adhesion between the awned grains, resulting in a higher friction for awned grains. The study showed that de-awning decreased such frictional properties as angle of repose and coefficient of friction of paddy. Awns tend to cling to each other, bridge-over and cause grain to adhere in a mass, resulting difficulty on the flowing of grains through the orifice of the paddy hopper [1].

Hulling index: Effect of de-awning and moisture content on husking index is presented in Fig. 4. The husking index of awned and de-awned paddy of Hashemi variety ranged

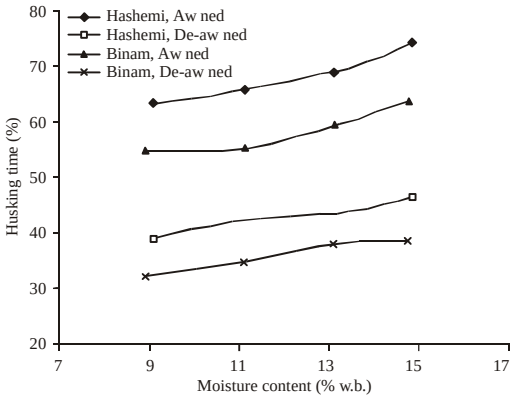

Fig. 3: Effect of de-awning and moisture content on husking time of paddy in rubber-roll husker for Hashemi and Binam varieties

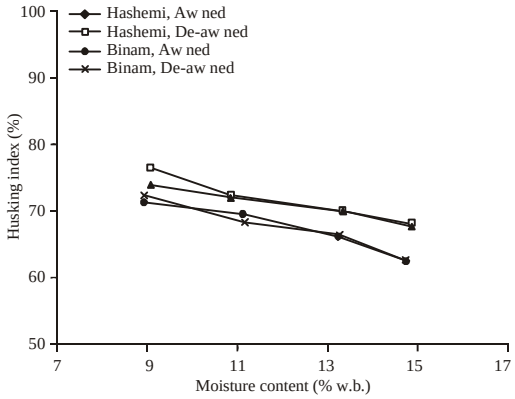

Fig. 4: Effect of de-awning and moisture content on husking index of paddy in rubber-roll husker for Hashemi and Binam varieties

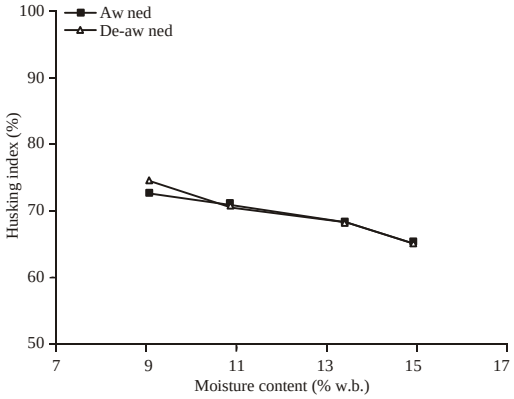

Fig. 5: Effect of de-awning and moisture content on husking index of paddy

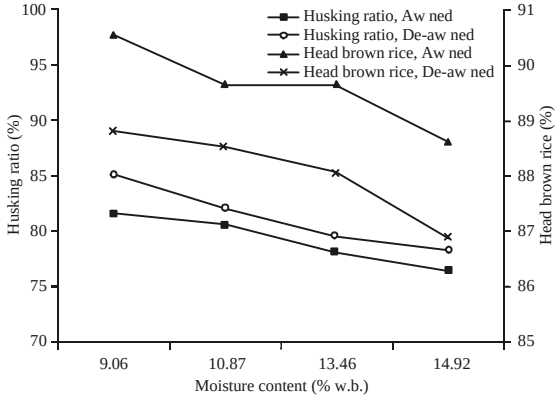

Fig. 6: Husking ratio and head rice of awned and de-awned paddy after husking in rubber-roll husker

from 73.88 to 67.71% and 76.59 to 67.93%, respectively as moisture content increased from 9.06 to 14.92% w.b. It varied for Binam variety from 71.40 to 62.43% and 72.27 to 62.35%, respectively as moisture content increased from 8.92 to 14.78% w.b. Statistical analysis showed that the effect of de-awning on husking index was nonsignificant wheras the effect of moisture content was significant at 1% level. The interaction effects of the independent variables were not significant. (Table 1). The mean values of husking index for awned and de-awned paddy decreased from 72.64 to 65.07% and 74.43 to 65.14%, respectively with increase of moisture content from 9.06 to 14.92% w.b. (Fig. 5). It was seen that moisture content was inversely related to husking index which decreased with increasing moisture content both for awned and de-awned paddy. The minimum husking index at higher moisture content could be attributed to the decreased husking ratio as well as head rice (Fig. 6). At higher moisture content, hardness of grains decreased which resulted in more breakage [4, 11].

CONCLUSIONS

Results of the study revealed that grain moisture content and de-awning had significant effects on husking quality of paddy varieties studied in the rubber-roll husker. Higher husking ratio and husking index were observed for de-awned paddy at lower moisture content levels. Results also indicated a decreasing trend in husking time with decreasing moisture content. The husking time of awned paddy was significantly higher than that of de-awned paddy at each level of moisture content. Due to the high adhesion friction of the awned paddy and consequent arching and clogging problems

through hopper orifice of the husker, de-awning of the paddy would be necessary for facilitating the grain flow and maintaining reliable performance of the machine.

REFERENCES

1. Alizadeh, M.R., S. Minaei, T. Tavakoli and M.H. Khoshtaghaza, 2006. Effect of de-awning on physical properties of paddy. Pak. J. Biol. Sci., 9: 1726-1731.
2. Rahman, A.M., M.K. Miah and A. Ahmed, 1996. Study of rice processing technology in Bangladesh, Agricultural Mechanization in Asia, Africa and Latin America, 27: 46-50.
3. Kunze, O.R., 1985. Effect of variety and environmental factors on milling qualities of rice. In: Rice grain quality and marketing, IRRI, Losbanos, Philippines.
4. Firozi, S. and M.R. Alizadeh, 2003. Study of factors affecting rice grain breakage during milling process. Final Research Report, Azad University of Rasht, Iran, pp: 111.
5. Payman, M.H., T. Tavakoli and S. Minaei, 1999. Determination of appropriate clearance between rolls in rubber-roll huskers in milling operations of three common rice varieties in Guilan. J. Agric. Sci., 5: 37-48.
6. Sacilik, K., R. Ozturk and R. Keskin, 2003. Some physical properties of hemp seed. Biosys. Eng., 86: 191-198.
7. Yang, W., T.J. Siebenmorgen, T.P.H. Thielen and A.G. Cnossen, 2003. Effect of glass transition on conductivity of rough rice. Biosys. Eng., 84: 193-200.
8. Farouk, S.M. and M.N. Islam, 1995. Effect of parboiling and milling parameters on breakage of rice grains. Agricultural Mechanization in Asia, Africa and Latin America, 26: 33-38, 45.
9. Roohi, 2002. The study on energy consumption during husking and whitening of three common paddy varieties in Guilan Province. Ms. thesis, Department of Agricultural Machinery Engineering, Tarbiat Modares University, pp: 131.
10. Singh, U.N., 1982. Performance and potential of rubber rolls husking paddy. Agricultural Mechanization in Asia, Africa and Latin America, Spring.
11. Ancheta, C.J. and S.C. Aandales, 1990. Total milled and head rice recoveries of paddy as influenced by its physico-varietal characteristics. Agricultural Mechanization in Asia, Africa and Latin America, 21: 50-54.

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 06-15, 2007
ISSN 1818-6769

Comparative Analysis of Amino Acid Between Transgenic and non Transgenic Egyptian Cotton (*Gossypium barbadense*) Lines under Different Salt Stress Conditions

[1]Osama A. Momtaz, [1]Ashraf H. Fahmy, [1]Ayman A. Diab and [2]A.H. Hanafy Ahmed

[1]Agricultural Genetic Engineering Research Institute, Agricultural Research Center, Giza, Egypt
[2]Department of Agricultural Botany, Plant Physiology Division,
Faculty of Agriculture, Cairo University, Egypt

Abstract: Based on transgenic cotton lines carrying the bacterial mtlD gene and non-transgenic, conventional cotton variety, a greenhouse experiment was conducted to assess the effect of different levels of salt stress on the amino acid content of both transgenic and non transgenic seeds. The amino acid profile was determined by GC mass-spectrum. Transgenic and non-transgenic cotton seeds were grown to maturity under different concentrations of salt stress after determining the field capacity. Seeds obtained from transgenic and non transgenic plants showed increasing concentration of amino acids with increasing the level of salt stress. However, the transgenic cotton seeds accumulated significant amounts of amino acids compared with non-transgenic seeds. There were some differences in mean content of some individual amino acids between transgenic and non transgenic seeds, with some significant differences at higher level of salt stress. Amongst the amino acids that showed significant differences at high level of salt stress were Alanine, Proline, Glutamine, Asparagine and histedine. The difference in amino acid content between transgenic and non transgenic plants was also high for tryptophane, lucine and tyrocine under low level of salt stress. Accumulation of amino acids in seeds from both transgenic and non transgenic plants as a result of salt stress appeared to play an important role in the acclimation to salt stress of cotton plants. However, the higher accumulation of total and individual amino acid in the seeds obtained form transgenic lines under salt stress compared with non transgenic seeds may be a result of the expression of the mtlD gene into the genome, which might prove that transferring the mtlD gene could be considered one of the effective strategies to produce salt tolerance cotton variety. The, results indicated that the insertion of mtlD gene in the transgenic cottons had some influence on the synthesis and accumulation of amino acids in transgenic seeds under salt stress.

Key words: Salt stress % amino acids % transgenic cotton % mtld gene % gc mass-spectrum % gossypium barbadense

INTRODUCTION

Salinity is the major stress factor, which delimit crop plans cultivation, especially in developing countries. The adverse effect of salinity on plants may lead to disturbances in plant metabolism, which consequently lead to a reduction of the plant growth and productivity [1, 2]. The ability of plants to cope with salinity stress is an important determinant of crop distribution and productivity in many areas, so it is important to understand the mechanisms that confer tolerance to saline environments. Many trials have been made to help the plants to overcome these disturbances using various treatments in the laboratory, for future application in the field.

Salt stress involves both osmotic stress, by limiting absorption of water from soil and ionic stress, resulting from high concentrations of potentially toxic salt ions within plant cells. A variety of protective mechanisms have evolved in plants to allow them to acclimatize to these unfavorable environmental conditions for survival and growth. Although the effect of salinity has been studied in a variety of plants, the mechanisms of salt tolerance are not well understood [3].

Biochemical studies have shown that plants under salinity stress accumulate a number of metabolites, which

Corresponding Author: Dr. Ashraf H. Fahmy, Agricultural Genetic Engineering Research Institute, Agricultural Research Center, Giza, Egypt

are termed compatible solutes because they do not interfere with biochemical reactions [4, 5]. These metabolites include carbohydrates, such as mannitol, sucrose and raffinose oligosaccharides and nitrogen-containing compounds, such as amino acids and polyamines. The function of compatible solute accumulation is often associated with osmotic adjustment, by lowering the water potential to improve the uptake of water against the external gradient. It is well established that the intracellular accumulation of these solutes prevents water loss and maintains the turgor pressure of the cell essential for the cell growth. However, a number of other roles for these compounds have been hypothesized [6-8].

Amino acids have been shown to accumulate in plants grown under salinity [9-13]. These amino acids include proline, arginine, alanine, glycine, serine, leucine and valine. Among increased amino acids, proline accumulates in larger amounts compared with other amino acids. In many plant species a remarkable increase in proline content under salt stress was observed [14, 15].

Increased accumulation of amino acids in salt stressed plants could be due to protein degradation [16], or inhibition of protein synthesis [17], or decreases in amino acids and amide export [18], or could be due to growth inhibition of leaves [19]. Stress-induced protein degradation may be essential in providing amino acids for synthesis of new proteins suited for growth or survival under the modified conditions and also substrates for energy metabolism [20].

Plants vary greatly in their capacity to accumulate amino acids under salinity. When plants subjected to salt stress, salt tolerant species/genotypes accumulate more amino acids than sensitive ones [21-25]. Furthermore, contents of amino acids increases also during cell adaptation *in vitro* in cultures treaded with NaCl [26].

Bacterial genes, engineered into plants and resulting in accumulation of proline, mannitol and other osmoregulatory solutes can increase ability to tolerate salinity [27-29].

Cotton is an especially attractive crop for genetic engineering because of its worldwide importance as a crop plant [30]. Various areas of interest are receiving attention; including fiber quality modification, stress tolerance and herbicide and pest resistance [31]. In Egypt, cotton is considered one of the major fiber crops and an essential economic asset.

In previous study [28], a bacterial gene encoding mannitol-1-phospahate dehydrogenase was used in the transformation of two Egyptian cotton varieties (Giza 86 and Giza 87) by particle bombardment with the aim of producing an accumulation of the sugar alcohol mannitol.

The work reported here represents a contribution to this approach aiming at studying the effect of different salt stress levels on the amino acid content of transgenic and non transgenic cotton seeds, in an attempt to compare the transgenic salt tolerant cotton lines with the non-transgenic, conventional cotton plants in response to different concentrations of salt stress.

MATERIALS AND METHODS

Plant material: The effect of different salt stress conditions on the content of total and individual amino acid in cotton seeds was investigated using both transgenic and non-transgenic seeds (Giza 87).

Transgenic materials: Transgenic cotton (*Gpssypium barbadense*) seeds were obtained from gene expression and regulation technology lab-Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza. In a previous work, a bacterial gene, the mtlD that encodes mannitol-1-phosphate dehydrogenase, was used to transform an extra long staple cotton variety (Giza 87). The transformation was carried out by particle bombardment of mature, dissected embryos using the Bio-Rad PDS/1000/He gun and the expression of mtlD gene and mannitol accumulation were confirmed [28].

Non-transgenic materials: Non-transgenic conventional cotton seeds were obtained from the Cotton Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

Experimental design: Transgenic and non transgenic seeds were grown in pots (30 cm diameter and 30 cm height) containing a mixture of soil, peat and sand (1: 1: 1) with a field water capacity of`35%. Mechanical and chemical analysis of soil samples from each pot were performed according to the method of Black [32]. A Completely Randomized Design was used with four replicates per treatment. An individual pot containing one plant represented a replicate. The experiments were replicated twice and the data presented in this study represent the mean among them.

Fertilization: Fertilization was carried out according to the recommendation of Ministry of Agriculture; each pot received 2.2 g calcium superphosphate (15.5% P_2O_5) and

0.7 g potassium sulphate (48% K_2O) before planting and 3.0 g ammonium nitrate (33.5% N) before the first irrigation.

Salt treatment: A salt mixture consists of sodium chloride, calcium chloride and magnesium sulfate (1: 1: 0.5) was added to the soil to give the desired concentration of salt stress in ppl (0, 1000, 2000, 3000, 4000, 6000, 8000 and 10000). Pots were then irrigated with water to balance the water lost by evapotranspiration. Evapotranspiration was estimated by the pot weight loss before each irrigation and the water content of the soil was always adjusted to the field capacity. The average daily evapotranspiration from the pots was approximately 15% of field water capacity. Adding powder of salt mixture direct into the soil and adjusting water irrigation to the field capacity exhibits a real simulation of salt stress condition and prevents accumulation of salt in soil that could happens if soil is irrigated with different concentration of salt solutions.

Germination conditions: Transgenic and non-transgenic cotton seeds were grown to maturity in a greenhouse under the same conditions of temperature, light and humidity (28°C; 16/8 h day/night and 50-70% relative humidity) and seeds were collected from all plants and subjected to amino acid analysis, however, non-transgenic plants did not survive and no seeds were obtained under 10000 ppl salt stress.

Extraction and identification of amino acids: Extraction and identification of amino acids in seeds were performed according to the method of Gary [33].

A. Extraction of amino acid: 0.2 g of dried seeds were hydrolyzed by 6 M HCl then placed in oven at 110°C for 20 h. After filtrating the mixture and evaporating the supernatant to dryness, 10 ml distilled water were added and the pH was adjusted to 1.8. Few grams of cation exchange resin were added; the mixture was shaked for 10 min on mechanical shaker and then filtrated. Five ml of 10% ammonia solution were added to cation exchange and shaked then filtrated. The ammonia solution was evaporated to dryness under stream of N2 at 40°C.

B. Derivatization procedure: After extraction of amino acids the residue was mixed with 1 ml methanolic HCl reagent and incubated in oven for 30 min at 70°C then the residue was evaporated to dryness under stream of N2 at 40°C. Two hundred and fifty microliter triflouroaceticanhydrie were added and incubated in oven for 10 min at 140°C then the solvent was evaporated to dryness under stream of at 40°C. Fifty microliter of methanol were added just before injection into gas liquid chromatography apparatus (Hewlett-Pakard 59801) under the following conditions:

- Column: HP-5 M.S. [cross-linked 5% Phenyl Metheyl Silicone] 30×0.250 mm
- Carrier gas: Helium, at flow rate 0.90 ml min^{-1}
- Injector Temp. Program: 100°C/2 min (6°C/min) to 260°C/1 min
- Detector: Mass selective detector

Concentration of total and individual amino acids in cotton seeds from transgenic and non transgenic plants was estimated in (µg g^{-1}) for each salt stress treatment (Table 2 and Figs. 1&2) and the percentage of the difference in amino acid content between transgenic and non transgenic seeds was calculated (Fig. 3)

RESULTS

Mechanical and chemical analysis of the soil: The results of soil analysis are presented in Table 1. Clay was the most abundant element in the soil (37.6%) followed by sand (34.4%) and silt (28%). The total soluble salt was 0.17% and the calcium carbonate was 2.30. 18.2% of the soil was for Ca, Mg, Na, K, HCO_3, SO_4 and Cl collectively. The analysis was performed for soil samples from each pot to ensure the homogeneity of the soil used in this study along the different treatments. The amount of total soluble salt in soil (0.17%) did not appear to have significant effect on accumulation of extra salt in soil; however the homogeneity of soil in all pots assured same condition and salt concentration for all plants.

Analysis of individual amino acids: The content of the individual amino acids (µg g^{-1}) for both transgenic and

Table 1: Mechanical and chemical properties of the soil used for seed germination

Property	Value	Property	Value
Clay (%)	37.60	EC (ds m^{-2}) 1:5	0.54
Silt (%)	28.00	Ca meq l^{-1}	3.20
Sand (%)	34.40	Mg meq l^{-1}	2.00
Texture class	Clay loam	Na meq l^{-1}	2.00
Total soluble salts (%)	0.17	K meq l^{-1}	1.00
Organic matter (%)	0.31	HCO3 meq l^{-1}	1.20
Calcium carbonate (%)	2.30	SO4 meq l^{-1}	4.50
pH	7.70	Cl meq l^{-1}	4.30

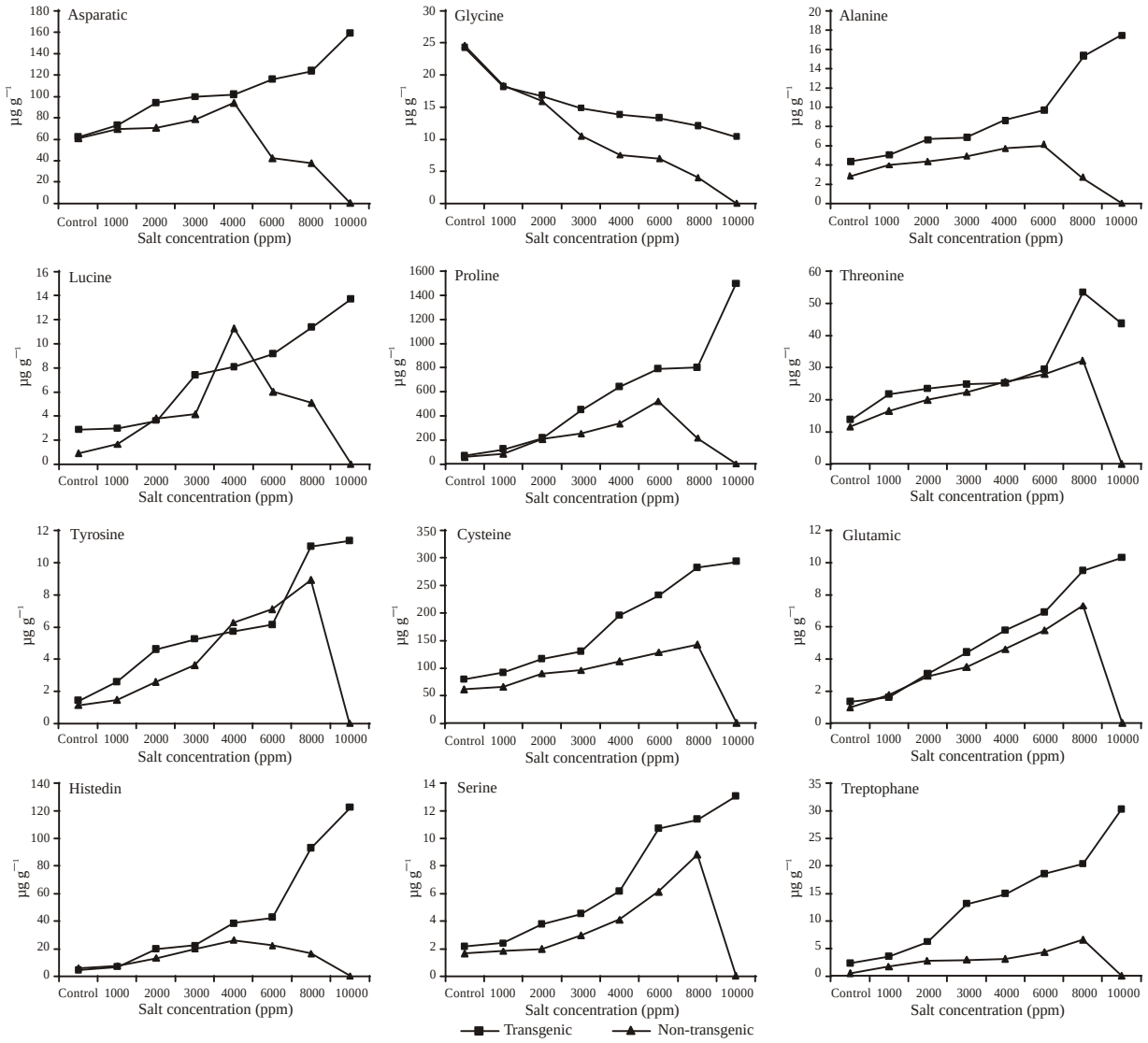

Fig. 1: Amino acid contents (µg g^{-1}) of transgenic and non transgenic cotton seeds obtained from plants grown under different salt stress condition

non transgenic seeds obtained from plants grown under different salt stress conditions is presented in Table 2 and (Fig. 1). In non transgenic seeds, some amino acids (Thr, Tyr, Cys, Glu, Tre and Sei) increased with increasing the salt concentration from 0 to 8000. In transgenic seeds these amino acids also increased with increasing salt concentration, however, the content of these amino acids was higher in transgenic seeds (Fig. 1).

The content of Asp and Luc increased with increasing salt stress from 0 to 4000 ppl then a dramatic decrease was observed at 6000 and 8000 ppl. This was not the case for transgenic seeds, where the content of Luc continued to increase to 1.5 fold and 2.2 fold at 6000 and 8000 ppl, respectively and Asp increased to 2.7 fold and 3.7 fold at 6000 and 8000 ppl, respectively.

The content of Ala and Pro in non transgenic seeds increased with increasing salt stress from 0 to 6000 ppl then decreased at 8000 ppl. While in transgenic seeds, the content of Ala and Pro continued to increase to 5.68 fold and 3.76 fold at 8000 ppl, respectively.

The content of Gly in transgenic and non transgenic seeds decreased with the increasing of salt stress from 0-8000 indicating that Gly is the most negatively affected amino acid during salt stress or it has no significant role in salt tolerance.

Table 2: Concentration (µg gG¹) of amino acids in cotton seeds from Transgenic (T) and non transgenic (C) plants grown under different concentrations of salt stress (ppm)

Salt	0		1000		2000		3000		4000		6000		8000		10000	
Con.	----------		----------		----------		----------		----------		----------		----------		----------	
(ppm)	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C*	T
Asp.	60.10	61.90	69.50	72.90	69.90	93.30	78.10	98.90	93.60	100.80	41.20	114.70	37.40	122.60	0	158.30
Gly.	24.50	24.20	18.30	18.00	15.80	16.80	10.50	14.80	7.58	13.72	6.91	13.23	4.01	12.09	0	10.27
Ala.	2.80	4.30	3.90	5.10	4.30	6.60	4.90	6.90	5.70	8.70	6.00	9.60	2.70	15.30	0	17.40
Luc.	1.00	2.80	1.60	3.00	3.80	3.70	4.20	7.40	11.30	8.10	6.00	9.20	5.10	11.40	0	13.80
Pro.	54.10	66.40	86.90	122.00	200.00	213.00	251.00	446.00	338.00	639.70	522.70	793.50	213.00	801.10	0	1501.00
Thr.	11.60	13.40	16.30	21.70	20.00	23.40	22.20	24.60	25.30	24.97	27.86	29.15	31.90	53.37	0	43.75
Tyr.	1.30	1.60	1.70	3.00	3.00	5.40	4.20	6.10	7.30	6.70	8.30	7.20	10.40	12.90	0	13.20
Cys.	60.20	80.20	65.40	91.30	88.80	116.00	95.60	130.00	111.00	194.40	128.90	232.10	141.00	283.10	0	293.20
Glu.	0.95	1.30	1.80	1.60	2.90	3.10	3.50	4.40	4.60	5.80	5.80	6.90	7.30	9.50	0	10.30
His.	5.90	3.80	7.10	6.70	13.10	19.70	19.60	21.70	26.30	38.20	21.80	42.70	16.50	93.00	0	122.40
Ser.	1.60	2.13	1.80	2.30	1.90	3.70	2.90	4.50	4.10	6.10	6.10	10.70	8.80	11.30	0	13.00
Tryp.	0.47	2.34	1.67	3.38	2.58	6.17	2.81	12.98	3.06	14.93	4.30	18.45	6.43	20.44	0	30.41
Total	224.50	264.30	275.90	350.90	426.00	510.80	499.50	778.28	637.80	1062.10	785.87	1287.40	484.50	1446.10	0	2227.03

*Non transgenic plants grown under 10000 ppm salt did not survive and no seeds were obtained

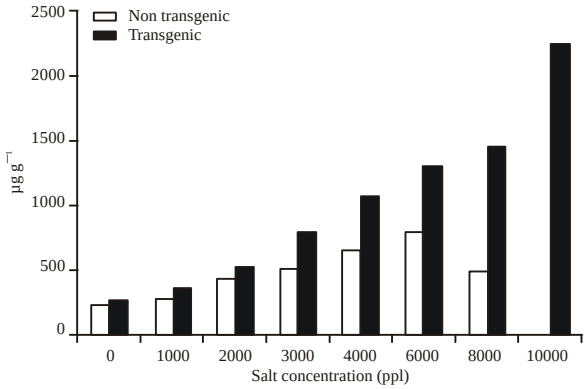

Fig. 2: Total amino acid content of transgenic and non transgenic cotton seeds obtained under different concentration of salt stress (0, 1000, 2000, 3000, 4000, 6000 and 8000 ppm)

At 10000 ppl salt stress, non transgenic plants did not survive and no seeds were obtained while transgenic plants survived and produced seeds with high level of amino acid content (Table 2). This result suggests that the over expression of the bacterial mtlD gene in transgenic plants have a significant role in salt tolerant under high salt concentration (10000 ppl) due to mannitol accumulation and other amino acids. In general, transgenic seeds showed more accumulation of individual amino acids compared with non transgenic seeds under different salt stress conditions. There were some differences in mean content of some amino acid between the transgenic and non-transgenic seeds, with some significant differences at higher level of salt stress. Amongst the amino acids that showed significant increase at 8000 ppl salt stress were Ala (5.68 fold) then followed by His (5.63 fold), Pro (3.7 fold), Asp (3.27 fold), Try (3.17 fold), Gly (3 fold) (Table 2 and Fig. 1). The 3.7 fold increase in proline concentration found in this study is in the range of values reported for other species (summarized by Delauney and Verma [34]).

Analysis of total amino acid: The data for total amino acids concentration in transgenic and non transgenic seeds were consistent with those for individual amino acids. The total amino acids, as determined by summation, progressively increased in transgenic and non transgenic seeds as the salt level increased (Table 2). However, the transgenic seeds accumulated significantly amino acids in seeds compared with non-transgenic plants. The total amino acid content was slightly higher in transgenic seeds compared with non transgenic seeds at low salt concentrations (0, 1000, 2000, 3000 ppl) and clearly built up in transgenic seeds at high salt concentrations (4000, 6000, 8000 ppl) (Fig. 2).

DISCUSSION

Indeed, transgenic seeds showed higher concentration of amino acids compared with non transgenic seeds. It is reported that salt stress provokes reduction in nitrate assimilation process, which supplies

ammonia for synthesis of new amino acids mainly via the GS/GOGAT pathway [35]. Furthermore, salinity inhibits drastically the protein synthesis in the plant tissues [36]. On this basis, the amino acids content is a consequence, at least in part, of the balance between these two processes. Moreover, the salt stress can induce an increment in the de novo synthesis of some particular amino acid [37].

In this study, Ala, His, Glu and Pro were accumulated largely in transgenic seeds compared with non transgenic seeds. These results agree with the findings of Silveira *et al*. [37] who reported a high accumulation of these amino acids in salt tolerant cowpea plants compared with salt sensitive plants under salt stress. It is reported that when plants subjected to salt stress, salt tolerant species/genotypes accumulate more amino acids than sensitive ones [21-25]. Significant differences in Asp, Glu, Pro, Arg, contents were found between the salt-tolerant *Triticum aestivum* L. cv. Sakha and the salt-sensitive *T. aestivum* cv. Regina [38]. The effect of salt stress on the amino acid content of rice varieties differing in salt tolerance is also investigated [39]. Amino acids were differentially accumulated according to the degree of salt tolerance and a relationship between changes in the concentration of amino acids and the accumulation of toxic ion Na+ were identified. This accumulation might be a consequence effect of salt stress on nitrogen metabolism in the plant, probably due to an increase of protein degradation [40, 41] and an inhibition of protein synthesis [42, 43]. The same result and conclusion were obtained for sorghum genotypes differ in their ability to tolerate salt stress [44] and two legumes varieties differing in salt tolerance [45]. Ashraf and Fatima [46] also reported that salt-tolerant accessions of safflower (*Carthamus tinctorius* L.) accumulated significantly greater amino acids in the leaves than the salt-sensitive accessions.

In some species, however, both salt tolerant and sensitive genotypes have similar concentration of some amino acids [10]. This was clearly demonstrated in this study for Asp, His and Glu where their content was almost the same in both transgenic and non transgenic seeds under law salt stress condition (0 and 1000 ppl). Supporting the view that no change occurs in protein synthesis or degradation when salt accumulation is mild [47].

Negative correlation between amino acid content and salt tolerant has been shown (e.g. in soybean and blackgram by Moftah and Michel [48], Ashraf [49], Ashraf [14]. These findings support our results that showed a decrease of the content of Gly in both transgenic and non transgenic seeds with increasing salt concentration from 0 to 8000 ppl. Catello *et al*. [50] reported a decrease in Gly content in Spanish plants grown under sever salt stress condition. This decrease is due to the usage of Gly in the synthesis of glycine betaine, which is common in salt stressed plants. Accordingly, Catello *et al*. [50] suggested that glycine betaine substituted Gly as an osmolyte in salt stressed tissues. Although there was a decrease in Gly content in both transgenic and non transgenic seeds, the content of this amino acid was higher in transgenic seeds compared with non transgenic. This could be explained as the transgenic seeds accumulated more Gly to be used in synthesis of more glycine betaine as an adaptive mechanism for salt tolerant.

Comparing the content of individual amino acids, five amino acids with quantitative importance, namely Ala, Pro, Glu, Asp and His showed a significant increase with salinity, especially in transgenic seeds, at high level of salt stress. The increase of Ala suggests that glycolysis and thus, respiration were increased to sustain the higher energy demand to confine salt to the vacuoles and to furnish carbon skeletons for the photorespiratory cycle [51].

The accumulation of both Pro and Glu in response to salinity is well established in literature (reviewed by Delauney and Verma [34]. Their accumulation is caused by both the activation of its biosynthesis from glutamate and by inactivation of its degradation [52]. However, Pro was shown to minimize cellular damage by enhancing the stability of proteins and membranes [53]. Thereby, it can be of protective value also at the low concentration of salt used in this study. A number of results was published which support the hypothesis of a positive correlation between the ability for Pro accumulation and the degree of salt tolerance [34, 54-56]. Proline accumulation may be interpreted as a symptom of injury caused by stress [57] or some type of adaptive response [58]. Osmoregulation has been attributed to Pro accumulation in tissues of the plants in response to salt stress [59]. On the other hand, Pro would stabilize enzymes as RUBISCO, allowing its efficient functioning even in the presence of NaCl [60].

The amino acid Asp has been shown to accumulate in a number of salt tolerant species (reviewed by Rabe [5]) and in some cases it is the most commonly accumulating nitrogen containing compounds that contain at least two amino groups, this suggest that it may be preferentially synthesized in response to stress and serve as important nitrogen sources for metabolic pathways.

Mansour [61] reported that accumulation of nitrogen containing compounds, especially amino acids, is usually correlated with plant salt tolerance. In our experiment, transgenic seeds responded to increasing salinity merely with double to triple fold increase of total and some individual amino acids especially at high level of salt stress. This variation might be a result of the over expression of the bacterial gene mtlD and the accumulation of mannitol in transgenic seeds used in this study. As found for wheat [27], tobacco [29], cotton [28] and other species, expression of the mtlD gene increased the tolerance level for salt stress due to accumulation of mannitol. On the other hand, the results obtained in this study suggest that there might be a correlation between mannitol accumulation and amino acid accumulation under critical salt stress conditions as we believe. It is reported that amino acid accumulation under salt stress need amount of carbon which may be a limiting resource in plants in saline environment, because of stomatal closure which reduces the flux of CO_2 to the leaves [62, 63]. Mannitol is a sugar alcohol, which is an intermediate of carbohydrate metabolism [64]. Many studies suggested physiological roles of sugar alcohols including osmoregulation and storage of reduced carbon and energy [65], service as compatible solutes [66], regulation of coenzymes [65, 67] and neutralization of hydroxyl radicals [8]. These studies support our suggestion that mannitol accumulation have a role in the significant accumulation of amino acids in transgenic seeds compared with non transgenic seeds under different salt stress conditions

Transgenic cotton varieties have provided new tools for abiotec stress tolerance but raised concerns about the relative performance of these varieties compared to conventional varieties. This work presents a comparative analysis of amino acid between transgenic and non transgenic Egyptian cotton lines under different salt stress conditions, in an attempt to investigate the performance of transgenic lines compared to conventional variety under salt stress.

Our results support the hypothesis that accumulation of amino acids is one of the adaptive mechanisms for salt stress condition. From the results obtained in this study, we suppose that transgenic seeds were more tolerant to different salt stress condition, specially at high level of salt, due to over expression of mtlD gene and accumulation of mannitol, which might play an important role in accumulation of amino acids. We conclude that transgenic seeds accumulated larger amount of individual and total amino acids compared with non transgenic seeds. Therefore, transgenic seeds have better chance of tolerating salt stress than non transgenic seeds. This conclusion is clearly demonstrated at high level of salt stress applied in this work.

Despite the evidences that transgenic seeds showed higher accumulation of individual and total amino acids compared with non transgenic seeds under different salt stress conditions and survived at 10000 ppl salt stress level while non transgenic seeds did not, further studies are necessary to compare the performance of transgenic and non transgenic cotton seeds under salt stress such as hormones content, growth and some other biochemical characteristics.

REFERENCES

1. Greenway, H. and R. Munns, 1980. Mechanism of salt tolerance in non-halophytes. Ann. Rev. Plant Physiol., 31: 149-190.
2. Sharma, P.K. and D.O. Hall, 1991. Interaction of salt stress and photoinhibition on photosynthesis in barley and sorghum. J. Plant Physiol., 138: 614-619.
3. Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert, 2000. Plant cellular and molecular responses to high salinity. Ann. Rev. Plant Physiol. Plant Mol. Biol., 51: 463-499.
4. Bohnert, H., D. Nelson and R. Jenson, 1995. Adaptations to environmental stresses. The Plant Cell, 7: 1099-1111.
5. Rabe, E., 1990. Stress physiology: the functional significance of the accumulation of nitrogen containing compounds. Hortic. Sci., 65: 231-243.
6. Vernon, D., M. Tarczynski, R. Jensen and H. Bohnert, 1993. Cyclitol production in transgenic tobacco. The Plant Journal, 4: 199-205.
7. Rabe, E. and C. Lovatt, 1984 De novo arginine biosynthesis in leaves of phosphorus-deficient Citrus and Poncirus species. Plant Physiol., 76: 747-752.
8. Smirnoff, N. and Q. Cumbes, 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry, 28: 1057-1060
9. Wyn Jones, R.G., 1981. Salt tolerance. In: Johnson, C.B., (Ed.). Physiological Processes Limiting Plant Productivity. Butterworth, London, pp: 271-292.
10. Rains, D.W., 1989. Plant tissue and protoplast culture: Applications to stress physiology and biochemistry. In: Jones, H.G., T.J. Flowers and M.B. Jones, (Ed.). Plants under Stresses. Biochemistry, Physiology and Ecology and their Application to Plant Improvement. Cambridge University Press, Cambridge, pp: 181-196.

11. Fathi-Ettai, R.A. and D. Prat, 1990. Variation in organic and mineral components in young seedlings under stress. Physiol. Plant., 79: 479-486.
12. Dubey, R.S. and M. Pessarakli, 1995. Physiological mechanisms of nitrogen absorption and assimilation in plants under stressful conditions. In: Pessarakli, M., (Ed.). Handbook of Plant and Crop Physiology. Marcel Dekker, New York, pp: 605-625.
13. Amonkar, D.V. and S.M. Karmarkar, 1995. Nitrogen uptake and assimilation in halophytes. In: Srivastava, H.S. and R.P. Singh, (Ed.). Nitrogen Nutrition in Higher Plants. Associated Publ. Co., New Delhi, pp: 431-445.
14. Ashraf, M., 1994. Breeding for salinity tolerance in plants. Crit. Rev. Plant Sci., 13: 17-42.
15. Ali, G., P.S. Srivastava and M. Iqbal, 1999. Proline accumulation, protein pattern and photosynthesis in regenerants grown under NaCl stress. Biol. Plant., 42: 89-95.
16. Becker, T.W. and H.P. Fock, 1986. The activity of nitrate reductase and the pool size of some amino acids and some sugars in water-stresses maize leaves. Photosynth. Res., 8: 267-274.
17. Dhindsa, R.S. and R.B. Cleland, 1975. Water stress and protein synthesis. Plant Physiol., 55: 781-788.
18. Tully, R.E., A.D. Hanson and C.E. Nelson, 1979. Proline accumulation in water stressed barley leaves in relation to translocation and the nitrogen budget-Plant Physiol., 63: 518-523.
19. Davies, W.J. and E. Van Volkenburgh, 1983. The influence of water deficit on the factors controlling the daily pattern of growth of Phaseolus trifoliates. J. Exp. Bot., 34: 987-999.
20. Raymond, P., R. Brouquisse, C. Chevalier, I. Couee, M. Dieuaide, F. James, D. Just and A. Pradet, 1994. Proteolysis and proteolytic activities in the acclimation to stress: The case of sugar starvation in maize root tips. In: Cherry, J.H. (Ed.). Biochemical and Cellular Mechanisms of Stress Tolerance in Plants. Springer-Verlag, Berlin, pp: 325-334.
21. Wyn Jones, R.G., J. Gorham and E. McDonnell, 1984. Organic and inorganic solute contents in the Triticeae. In: Staples, R.G. and G.H. Toenniessen, (Ed.). Salinity Tolerance in Plants. Wiley, New York, pp: 189-203.
22. Heuer, B., 1994. Osmoregulatory role of proline in water stressed plants. In: Pessarakli, M., (Ed.). Handbook of Plant and Crop Stress. Marcel Dekker, New York, pp: 227-246.
23. Dubey, R.S. and M. Rani, 1995. Influence of NaCl salinity on growth and metabolic status of protein and amino acids in rice seedlings. J. Agron. Crop Sci., 162: 97-106.
24. Dubey, R.S., 1997. Nitrogen metabolism in plants under salt stress. In: Jaiwal, P.K., R.P. Singh and A. Gulati, (Ed.). Strategies for Improving Salt Tolerance in Higher Plants. Oxford and IBH Publ. Co., New Delhi, pp: 129-158.
25. Ashraf, M., 1997. Improvement of salt tolerance in some native pulse crops. In: Jaiwal, P.K., R.P. Singh and A. Gulati, (Ed.). Strategies for Improving Salt Tolerance in Higher Plants. Oxford and IBH Publ. Co., New Delhi, pp: 413-433.
26. Hasegawa, P.H., R.A. Bressan and A.K. Handa, 1986. Cellular mechanisms of salinity tolerance. Hort. Sci., 21: 1317-1324.
27. Abebe, T., C. Arron, Guenzi Bjorn Martin and John C. Cushman, 2003. Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. Plant Physiol., 131: 1748-1755.
28. Momtaz, O.A., Ahmed Barakat, Ahmed Bahieldin, Mona Sadek and Magdy Madkour, 2001. Transformation of Egyptian Cotton Varieties Using a Bacterial mtlD Gene. Proceedings of the Beltwide Cotton Conferences, Cotton Improvement Conference, Anaheim, 9-13 January, CA, USA, 2: 1412-1422.
29. Mitchell, C. Tarczynski, Richard G. Jensen and Hans J. Bohnert, 1992. Expression of a bacterial mtlD gene in transgenic tobacco leads to production and accumulation of mannitol. Proc. Natl. Acad. Sci., USA, 89: 2600-2604.
30. Firoozabady, E., D.L. DeBoer, D.J. Merlo, E.L. Halk, L.N. Amerson, K.E. Rashka and E.E. Murray, 1987. Transformation of cotton (*Gossypium hirsutum* L.) by Agrobacterium tumefaciens and regeneration of transgenic plants. Plant Mol. Biol., 10: 105-116.
31. Pannetier, C., M. Giband, P. Couzi, V. Le Tan, M. Mazier, J. Tourneur and B. Hau, 1997. Introduction of new traits into cotton through genetic engineering: insect resistance as an example. Euphytica, 96: 163-166.
32. Black, A.L., 1982. Long-term N-P fertilizer and climatic influences on morphology and yield components of spring wheat. Agron. J., 74: 651-657.
33. Gary, A. Mabbott, 1990. Qualitative amino acids analysis of small peptides by GC/MS. J. Chem. Edu., 67: 441-445.

34. Delauney, A.J. and D.P.S. Verma, 1993. Proline biosynthesis and osmoregulation in plants. Plant J., 4: 215-223.
35. Gouia, H., M.H. Ghorbal and B. Touraine, 1994. Effects of NaCl on flows of N and mineral ions and on NO_3-reductase rate within whole plants of salt-sensitive beanand salt-tolerant cotton. Plant Physiol., 105: 1409-1418.
36. Lutts, S., V. Majerus and J.M. Kinet, 1999. NaCl effects on proline metabolism in rice (Ori a sati a) seedlings. Physiol. Plant., 105: 450-458.
37. Silveira, J.A.G., B.B. Cardoso, A.R.B. Melo and R.A. Vie´gas, 1999. Salt-induced decrease in nitrate uptake and assimilation in cowpea plants. Braz. J. Plant Physiol., 11: 77-82.
38. Livia Simon-Sarkadi1, Gábor Kocsy and Zoltn Sebestyén, 2002. Effect of salt stress on free amino acid and polyamine content in cereals. Proceedings of the 7th Hungarian Congress on Plant Physiology, 46: 73-75.
39. Nguyen Thi Thu Hoai, Ie Sung Shim, Katsuichiro Kobayashi and Usui Kenji, 2003. Accumulation of some nitrogen compounds in response to salt stress and their relationships with salt tolerance in rice (*Oryza sativa* L.) seedlings. Plant Growth Regulation, 41: 159-164.
40. Levitt, J., 1980. Salt and ion stresses. In: Levitt, J., (Ed.). Responses of Plants to Environmental Stresses. Academic Press, 2: 365-453.
41. Dubey, R.S. and M. Rani, 1990. Influence of NaCl salinity on the behaviour of protease, aminopeptidase and carboxypeptidase in rice seedlings in relation to salt tolerance. Aust. J. Plant Physiol., 17: 215-221.
42. Hurkman, W.J. and C.K. Tanaka, 1987. The effect of salt on the pattern of protein synthesis in barley roots. Plant Physiol., 83: 517-524.
43. Silveira, J.A.G., A.R.B. Melo, R.A. Vi egas and J.T.A. Oliveira, 2001. Salinity-induced effects on nitrogen assimilation related to growth in cowpea plants. Plant Sci., 46: 171-179.
44. Claudivan Feitosa de Lacerda, Jose´ Cambraia, Marco Antonio Oliva, Hugo Alberto Ruiz, Jose´ Tarquý´nio Prisco, 2003. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. Environ. Exp. Bot., 49: 107-120.
45. Ashraf Muhammad and Alweena Bashir, 2003. Salt stress induced changes in some organic metabolites and ionic relations in nodules and other plant parts of two crop legumes differing in salt tolerance. Flora, 198: 486-498.
46. Ashraf, M. and H. Fatima, 1995. Responses of salt-tolerant and salt-sensitive lines of safflower (*Carthamus tinctorius* L.) to salt stress. Acta-Physiologiae-Plantarum, 17: 61-70.
47. Robinson, S.A., G.R. Stewart and R. Philips, 1992. Regulation of glutamate dehydrogenase activity in relation to carbon limitation and protein catabolism in carrot cell suspension culture. Plant Physiol., 98: 1190-1195.
48. Moftah, A.B. and B.B. Michel, 1987. The effect of sodium chloride on solute potential and prolin accumulation in soybeans leaves. Plant Physiol., 83: 283-286.
49. Ashraf, M., 1989. The effect of of NaCl on water relations, chlorophyll, protein and proline content of two cultivars of blachgram (*Vigna mungo* L.). Plant Soil, 119: 205-211.
50. Catello Di Martin, Sebastiano Delfine, Roberto Pizzuto, Francesco Loreto and Amodio Fuggi, 2003. Free amino acids and glycine betaine in leaf osmoregulation of spinach responding to increasing salt stress. New Phytologist, 158: 455-463.
51. Lea, P.J., S.A. Robinson and G.R. Stewart, 1990. The enzymology and metabolism of glutamine, glutamate and asparagine. In: Miflin, B.J., (Ed.). The biochemistry of plants. New York, USA, Academic Press, pp: 121-169.
52. Yoshiba, Y., T. Kiyosue, K. Nakashima, K. Yamaguchishinozaki and K. Shinozaki, 1997. Regulation of levels of proline as an osmolyte in plants under water-stress. Plant Cell Physiol., 38: 1095-1102.
53. Csonka, L.N., 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev., 53: 121-293.
54. Khan, A.H., F.Y. Ashraf and A.R. Azmi, 1993. Osmotic adjustment in whea~a response to water stress. Pak.J. Sci. Ind. Res., 36: 151-155.
55. Nandwal, A.S., S. Bharti, M.S. Kuhad and J. Singh, 1993. Response of pigeon pea cultivars to water stress. Biol. Plant. (Prague), 35: 641-644.
56. Santos-Diaz, M.S. and N. Ochoa-Alejo, 1994. Effect of water stress on growth, osmotic potential and solute accumulation in cell cultures from chili pepper (a mesophyte) and creosote bush (a xerophyte). Plant Sci., 96: 21-29.
57. Riazi, A., K. Matsuda and A. Arslan, 1995. Water Stress-Induced Changes in Concentrations of Proline and Other Solutes in Growing Regions of Young Barley Leaves. J. Exp. Bot., 36: 1716-1725.

58. Aspinall, D. and L.G. Paleg, Proline Accumulation: Physiological Aspects, 1981. In The Physiology and Biochemistry of Drought Resistance in Plants; Paleg, L.G. and D. Aspinal, (Eds.). Academic Press: Australia, pp: 205-241.
59. De La Rosa, M. and R.K. Maiti, 1995. Biochemical Mechanism in Glossy Sorghum Lines for Resistence to Salinity Stress J. Plant. Physiol., 146: 515-519.
60. Solomon, A., S. Beer, Y. Waisel and L.G. Paleg, 1994. Effects of NaCl on the Carboxylating Activity of Rubisco from Tamarix jordanis in the Presence of Proline-Related Compatible Solutes. Physiol. Plant., 90: 198-204.
61. Mansour, M.M.F., 2000. Nitrogen containing compounds and adaptation of plants to salinity stress. Biologia plantrarum 43: 491-500.
62. Stoop, J.M.H., J.D. Williamson and D.M. Pharr, 1996. Mannitol metabolism in plants: A method for coping with stress. Trends Plant Sci., 1: 139-144.
63. Jefferies, R.L. and T. Rudmik, 1984. The response of halophytes to salinity: An ecological prespective. In: Staples, R.C. and G.H. Toenniessen, (Ed.). Salinint tolerance in plants. Wiley, New York, pp: 213-227.
64. Tarczynski, M.C., R.G. Jensen and H.J. Bohnert, 1992. Expression of a bacterial mtlD gene in transgenic tobacco leads to production and accumulation of mannitol. Proc. Natl. Acad. Sci., USA, 89: 2600-2604.
65. Lewis, D.H. and D.C. Smith, 1967. Sugar alcohols (polyols) in fungi and green plants. New Phytol., 66: 143-184.
66. Yancey, P.H., M.E. Clark, S.C. Hand, R.D. Bowlus and G.N. Somero, 1982. Living with water stress: Evolution of Osmolyte systems. Science, 217: 1214-1222.
67. Loescher, W.H., 1987. Physiology and metabolism of sugar alcohols in higher plants. Phsiol. Plant., 70: 553-557.

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 16-23, 2007
ISSN 1818-6769

Field Response of Groundnut (*Arachis hypogea L.*) Cultivars to Mycorrhizal Inoculation and Phosphorus Fertilizer in Abeokuta, South West Nigeria

M.O. Atayese

Department of Plant Physiology and Crop Production, University of Agriculture, Abeokuta, Nigeria

Abstract: A field experiment was conducted in Abeokuta, south western Nigeria, to evaluate the growth and yield response of three groundnut cultivars to inoculation with mycorrhizal fungus (*Glomus mosseae*) and phosphorus (P) fertilization in 2003 and 2004 planting seasons. The design was split-split plot in Randomized Complete Block Design (RCBD) in a 3×2×2 factorial combination of groundnut cultivars (RMP_{91}, RRB and RMP_{12}), phosphorus (54 and 0 kg haG^{1}) and mycorrhizal inoculation (inoculated and uninoculated)). Observations were made on canopy spread, leaf area, dry matter yield and grain yield. Mycorrhizal root infections, leaf P uptake and available P in the rhizosphere were also determined. Result shows that inoculation of groundnut cultivars with *G. mosseae* significantly enhanced grain yield in the 2003 planting season (54% in RMP_{91} to 66% in RMP_{12}) whereas the enhancement was lower and only significant in RRB (21%) in the 2004 planting season. Phosphorus fertilization enhanced grain yield by a range of 22% (RMP_{91}) and 40% (RMP_{12}) in the 2003 planting season while it was a range of between 20% (RMP_{91}) and 16% (RRP) in the 2004 planting season. Mycorrhizal root infection was as high as 64.1% relative to control (10.6%) in 2003 season. Inoculation with *G. mosseae* increased leaf P uptake by 30% within the two planting seasons. Phosphorus application increased the level of P in the leaf (average 32%}in the two years and rhizosphere soil P (average 1400%) only in the 2004 planting season. Percentage root colonisation ranged between 60 and 67% in all the inoculated plots in the two years. There was a marked increase in the root infection rate in the uninoculated plots (averaged 180%) in the 2004 planting season. In the 2004 planting season, available rhizosphere soil P averaged 2.06 mg kgG^{1} in the inoculated plots when compared to 2.6 mg kgG^{1} in P fertilized and 2.69 mg kgG^{1} in the P+M plots. All the treatments increased canopy spread (average 25%), leaf area (average 14%) and root dry weight (17%) over the control in the 2003 planting season, while in 2004 planting season, increase was recorded in canopy spread (average 22%), leaf area (average 40%) and shoot dry weight (average 35%) over the control. Inoculation of groundnut cultivars with the mycorrhizal fungus improved their performance in the field and compared favorably with the groundnuts fertilized with 54 kg haG^{1} of SSP fertilizer.

Key words: Groundnut cultivars % arbuscular mycorrhizal inoculation % leaf phosphorus uptake % grain yield % transitional agro-ecological zone

INTRODUCTION

Groundnut (*Arachis hypogea* L.) is comonly grown in the Northern Guinea Savanna of Nigeria. It was a crop that accounted for large percentage of Nigeria's export produce in the 1960s. Over the years, the production has gone drastically down owing to the increasing cost of production arising from inputs such as phosphorus fertilizers, labour and unpredictable environmental factors. Attempts are currently on to effectively include this crop in the farming systems of the southern ecologies [1, 2]. Nitrogen and phosphorus are important for effective production of the crop. Groundnut is a legume that requires phosphorous for growth and development, nitrogen fixation as well as nodule formation [3, 4]. Nitrogen need of the crop is sufficiently met through nitrogen fixation in most soils while inorganic fertilizers are applied to supply the phosphorus requirement. In most tropical soils, phosphorus deficiency is a major problem [5, 6]. High costs of fertilizers and

Corresponding Author: Dr. M.O. Atayese, Department of Plant Physiology and Crop Production, University of Agriculture, Abeokuta, Nigeria

logistic problem in distribution have made it difficult for small scale farmers to use them as at when needed. This has reduced hectarage and yield. There is also competing demands for fertilizers from more popular crops such as maize and rice in this region. However, Arbuscular Mycorrhizal (AM) fungi are known to improve water in drought stress conditions [7] and nutrient uptakes of plants particularly P in deficient soils [8]. Plants are also protected against some root pathogens [9]. These fungi are involved in symbiotic association with the roots of with more than 95% of plants [10, 11]. There are claims that the response of different plant species to mycorrhizal fungi inoculation varies considerably, even crops within same species but different cultivars may respond differently to mycorrhizal fungi [12]. These authors noted that improved cultivars of tomato (*Lycopersicum esculentum* Mill.) in a low P soil were more responsive to AM colonization than the wild type. Ironically, significant variation was also noticed among the wild accessions and even among the improved cultivars in responsiveness to mycorrhizal fungi colonization. Studies into the use of mycorrhizal fungi in improving crop production are still relatively very few in Nigeria. Most of the available trials have been conducted under partially or wholly controlled environments. The objective of this study therefore, is to compare the effects of an AM fungus (*Glomus mosseae*) and phosphorus on the growth and yield of three groundnut cultivars under field conditions in Abeokuta, a forest savannah transition zone of south west Nigeria.

MATERIALS AND METHODS

The experiment was conducted at the University of Agriculture Abeokuta Teaching and Research Farm, Alabata in south western Nigeria (70° 15'N, 30° 25'E). The site lies within the humid low land tropical region with two distinct seasons. The wet seasons extends from April to October while the dry season starts from November to March. The mean annual rainfall is 1113.1 mm, with a characteristic bimodal distribution which peaks in July and September with a break in August. Mean monthly temperature varies from 22°C in August to 36°C in March. The relative humidity ranges between 75% in February and 88% in July. The trial was carried out in the wet seasons of 2003 and 2004. Experimental soil characteristics is presented in Table 1 and was determined by collecting composite soil sample before planting for analysis using the standard procedures. The samples were air dried and sieved through a 2 mm mesh sieve before analysis. Particle size was determined by the pipette method, pH was measured in 0.01 M $CaCl_2$ at 1:2 soil : solution ratio, Organic carbon was determined by the Walkley-Black wet oxidation method [12]. The exchangeable cations were extracted in neutral ammonium acetate solution [14]. Ca and Mg were determined by the atomic absorption spectrophotometer and K by flame photometry. The total N was determined by the microkjeldahl method [15], available phosphorus was extracted by the Bray 1 exractant and P in solution determined by the Molybdate blue color method [16].

Inoculum preparation: Strains of *G. mosseae* was obtained from the culture stock of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria and multiplied on maize plants in green house plastic pots culture according to the procedure of Khalil *et al*. [17]. After three months, the maize plants whose inflorescence had been removed were allowed to dry to induce sporulation of the fungus. The crude inoculum which consisted of soil containing spores, hyphae and maize roots infected with the fungus contained about 500-650 spores per 100 g of soil.

Experimental procedures: The experimental design was split-split plot in RCBD in a 3×2×2 factorial combination of cultivars (RMP_{91}, RMP_{12} and RRB), Phosphorus (54 and 0 kg ha^{-1}) and Inoculation (with [M] or without [NM]) AM fungus. All treatments were replicated three times. Fifty grams of crude inoculum of *G. mosseae* was introduced into the planting holes of the inoculated plots (M) while the same quantity of attenuated inoculum was applied into the planting hole of non-inoculated plots (NM). All treatments were replicated three times. The planting space of groundnut was 75×25 cm with two seeds per hole and each plot measured 4×3 m. Single Super Phosphates (SSP) was applied basally in phosphorus fertilized plots. Measurement and observation of various parameters were taken at 100% flowering for plant height and root to shoot ratio. Leaf area was determined using the punch technique as described by Nichiporohvic [18]. Percentage root infection was determined according to the grid line intersect technique of Goivanetti and Mosse [19]. Roots and shoots weights (g $plant^{-1}$) were determined at harvest. Data were collected on shelling (%), pod number/plant, 100 seed weight (g) and grain yield (t ha^{-1}). Leaf P concentration (%) was determined according to molybdenum blue method of Murphy and Rilley [16] and

Table 1: Initial values of soil parameters at the beginning of the trials in the in 2003 and 2004 planting seasons in Abeokuta

Soil characteristics	2003	2004
Sand (%)	82.20	82.00
Silt (%)	9.30	9.40
Clay (%)	8.50	8.60
Textural class	Sandy	Sandy
CEC (cmol kg^{-1})	1.31	1.34
Organic carbon (g kg^{-1})	18.60	19.60
pH ($CaCl_2$)	4.80	5.00
Available P (mg kg^{-1})	4.92	4.95
Total N (mg kg^{-1})	1.16	1.16

leaf P uptake was subsequently calculated as the sum of its concentrations and the dry weight. Data were subjected to statistical analysis using Analysis of Variance (ANOVA) and means were separated using Least Significant Difference (L.S.D).

RESULTS

Soil characteristics: Table 1 shows the initial soil data before commencement of the experiment in the first and second planting seasons. The soil was sand and acidic in reaction with low phosphorus, nitrogen and moderate level of organic carbon.

Growth and yield response: In 2003, inoculation and P fertilization individually and in combination increased canopy cover in the groundnut cultivars except for RMP_{91}. In all the cases, the cover was similar but higher than the control (uninoculated and without phosphorus) (Table 2). Similarly, root dry weight was significantly higher and comparable in all the treatments than the control. Shelling percentage and 100 seed weight were also improved by either phosphorus fertilization, inoculation or both. In a similar manner, shoot dry weight and number of pods per plant was increased by phosphorus or AM inoculation just as the number of pods per plant was generally observed to be higher in RRB than in the other two cultivars (Table 2). In 2004 planting season, canopy spread was also increased by phosphorus fertilization and inoculation singly or in concert in all the cultivars and this was generally observed to be higher than the values obtained in 2003 (Table 2). Similarly, phosphorus fertilization and inoculation enhanced root and shoot dry weights (Table 2). Phosphorus and AM inoculation individually or in combination increased leaf area (25%), root (8%) and shoot dry weights (43%) in all the three cultivars.

Grain yield: Phosphorus fertilization and AM inoculation increased the grain yield of groundnut cultivars in 2003 and only in RRB in 2004 planting seasons (Table 2a & b). The percentage enhancement was higher in 2003 than in 2004 planting seasons. While the grain yield increase averaged 90% in 2003, it was reduced to 55% in 2004 season. However, there was about 60% increase in grain yield in the control plot in 2004 season whereas it was relatively stable in all other treatments (Table 2a & b). In 2004, grain yield of RMP_{91} and RMP_{12} were not significantly different from each other.

Table 2a: Effect of *Glomus mosseae* inoculation and phosphorus on growth and yield parameters of three groundnut varieties in 2003

Variety	Treatment	Canopy spread (cm^2)	Leaf area (m^2)	Root dry weight (t ha^{-1})	Shoot dry weight (t ha^{-1})	Shelling (%)	No. of pod plant^{-1}	100 seed weight/plant (g)	Grain yield t ha^{-1}
RMP_{91}	P	13.84	0.50	2.60	10.48	74.60	77.00	57.40	0.53
	M	13.73	0.42	2.35	10.62	74.58	100.00	57.02	0.64
	P+M	15.63	0.46	2.32	10.70	76.20	99.00	57.95	0.76
	Control	11.86	0.35	2.11	8.87	68.54	97.00	50.66	0.41
RRB	P	15.70	0.53	2.60	9.87	75.06	114.00	53.90	0.66
	M	13.70	0.51	2.57	10.87	72.84	124.00	51.78	0.77
	P+M	15.16	0.53	2.32	10.89	75.60	114.00	54.57	0.84
	Control	11.94	0.43	2.10	9.55	68.70	104.00	49.71	0.55
RMP_{12}	P	14.99	0.42	2.17	7.80	71.20	82.00	56.88	0.66
	M	15.29	0.44	2.27	10.24	71.22	80.00	55.70	0.66
	P+M	17.21	0.44	2.18	11.79	74.43	76.00	57.39	0.78
	Control	11.72	0.33	1.94	8.32	68.92	68.00	51.90	0.4
	$L.S.D_{0.05}$	2.09	0.05	0.10	1.38	4.57	39.00	3.82	0.11

Abbreviation: P-Phosphorus, M-Inoculated, Control-No Phosphorus and no inoculation

Table 2b: Effect of *Glomus mosseae* inoculation and phosphorus on growth and yield parameters of three groundnut varieties in 2004 planting seasons

Variety	Treatment	Canopy spread (cm^2)	Leaf area (m^2)	Root dry weight (t ha^{-1})	Shoot dry weight (t ha^{-1})	Shelling (%)	No. of pod $plant^{-1}$	100 seed weight/plant (g)	Grain yield t ha^{-1}
RMP_{91}	P	23.00	0.74	2.31	19.99	73.07	95.00	57.03	0.64
	M	21.67	0.66	2.39	16.75	72.74	90.00	55.32	0.67
	P+M	23.33	0.67	2.41	22.50	71.36	78.00	58.59	0.66
	Control	18.11	0.51	2.27	14.04	70.27	76.00	52.08	0.66
RRB	P	23.56	0.75	2.31	22.60	68.70	98.00	50.93	0.74
	M	21.71	0.8	2.41	18.99	72.52	111.00	50.07	0.74
	P+M	22.70	0.78	2.41	25.24	68.71	77.00	51.96	0.76
	Control	21.02	0.58	2.11	15.08	66.62	118.00	49.07	0.61
RMP_{12}	P	26.08	0.7	2.14	23.92	70.01	80.00	58.3	0.69
	M	23.11	0.73	2.36	18.21	70.88	74.00	52.06	0.66
	P+M	25.63	0.8	2.32	25.84	70.09	88.00	56.27	0.71
	Control	22.11	0.69	2.14	15.25	67.42	75.00	50.36	0.67
	$L.S.D_{0.05}$	2.42	0.05	0.16	0.98	6.90	29.00	5.78	0.04

Abbreviation: P-Phosphorus, M-Inoculated, Control-No Phosphorus and no inoculation

Table 3: Effect of *Glomus mosseae* inoculation and phosphorus on root to shoot ratio in 2003 and 2004 planting seasons

Variety	Treatment	Root to shoot ratio (2003)	Root to shoot ratio (2004)
RMP_{91}	P	0.25	0.22
	M	0.22	0.24
	P+M	0.22	0.21
	Control	0.24	0.25
RRB	P	0.25	0.24
	M	0.24	0.22
	P+M	0.23	0.23
	Control	0.21	0.23
RMP_{12}	P	0.28	0.21
	M	0.24	0.22
	P+M	0.2	0.22
	Control	0.22	0.21
$L.S.D_{0.05}$		0.05	0.05

Abbreviation: P-Phosphorus, M-Inoculated, Control-No Phosphorus and no inoculation

Mycorrhizal infection: In 2003, phosphorus fertilization significantly reduced root AM infection in all the groundnut cultivars (values ranged between 8-12%). Whereas development of infection in inoculated plots as well as plots fertilized with phosphorus was significantly higher (60-65%) than control plots (10-12%) (Fig. 1a). Similar observations were recorded in the two seasons of the experiment (Fig. 1a & b). There were significant differences among cultivars inoculated with mycorrhizal fungi, those fertilized with phosphorus and control in both years. Plot fertilized with phosphorus had about 14% root colonization of the inoculated plots in all cultivars. The control plots without phosphorus fertilization had average of about 11% infection in 2003 planting season with a similar trend in 2004 planting season. A major deviation was the increase in the percentage of infection recorded for control plots which was about 210% of what was obtainable in the 2003 planting season (Fig. 1a & b).

Root to shoot ratio, P uptake and phizosphere P: Root to shoot ratio was not significantly influenced by either phosphorus fertilization or mycorrhizal inoculation in all the groundnut cultivars in the two planting seasons (Table 3). Leaf phosphorus uptake was enhanced by phosphorus fertilization, mycorrhizal inoculation and both when compared to all the control plots in the two planting seasons (Fig. 2a & b). In all the cultivars, phosphorus uptake was significantly enhanced by 155% on the average. Rhizosphere available phosphorus was increased by phosphorus and mycorrhizal inoculation singly or in combination in all the groundnut cultivars only in the 2004 planting season (Fig. 3a & b). Plot with P fertilization [P] has the highest available phosphorus (1400%) among the three cultivars, followed by the plots under AM inoculation and P fertilization [P+M] (930%) and the inoculated plots [M] (300%) of the control in RMP_{91}. The range of enhancement was similar in RRB and RMP_{12} (Fig. 3a & b).

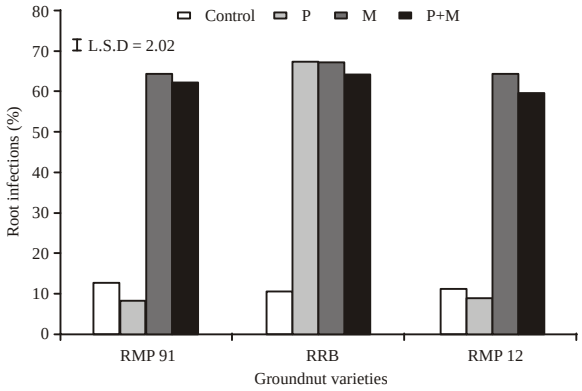

Fig. 1a: Effect of *Glomus mosseae* inoculation and phophorus on root infection in 2003 planting season

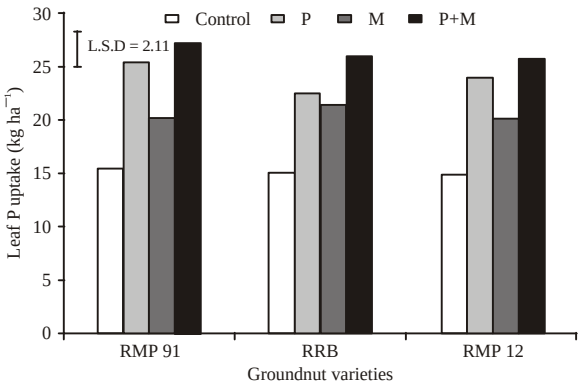

Fig. 2a: Effect of *Glomus mosseae* inoculation and phosphorus on leaf P uptake in 2003 planting season

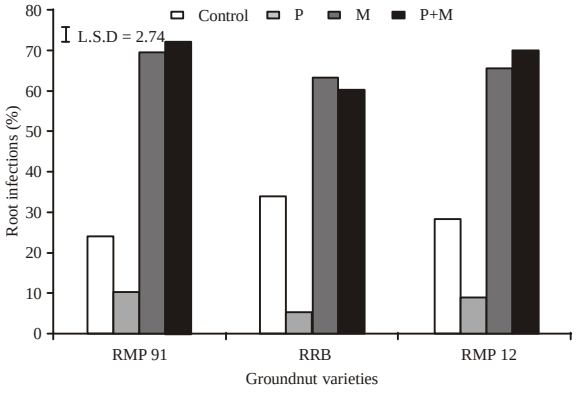

Fig. 1b: Effect of *Glomus mosseae* inoculation and phophorus on root infection in 2004 planting season

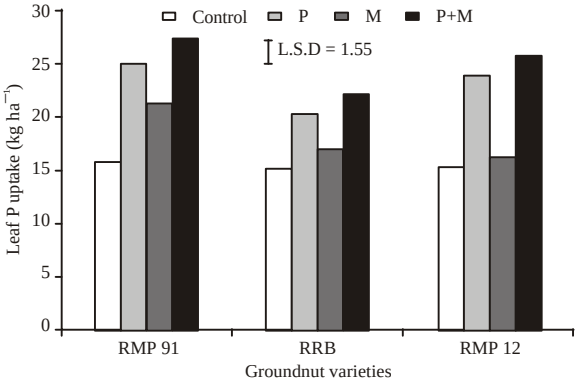

Fig. 2b: Effect of *Glomus mosseae* inoculation and phosphorus on leaf P uptake in 2004 planting season

DISCUSSION

The trial site is low in phosphorus and nitrogen but moderate in organic carbon. These are optimum conditions for establishment of mycorrhizal associations with plant roots [4]. The infection results of this experiment confirm the findings of Simpson and Daft [20] on the ability of groundnut to form mycorrhizal association particularly with *Glomus* sp. It also showed that *G. mosseae* can compete favourably among the varieties of other soil fungi, if it is present in reasonable quantity. Inoculation of groundnut with the fungus brought about enhancement in the general growth and the eventual improvement in the economic yield. This growth enhancement is due to potential of the fungus to improve water and phosphorus uptake as suggested by Harley and Smith [21] and Sieverding [22]. In addition, nitrogen fixation could have been enhanced by the fungi as reported by Simpson and Daft [19]. This is possible since the field was not nitrogen fertilized [23]. Osonubi *et al*. [24] had also reported an increase in phosphorus uptake in alley cropped cassava in a degraded alfisol after inoculation with *G. deserticola*. The behavior of the rhizosphere phosphorus as obtained in this study supports this assertion. The infection in the first year was similar to the second year in all the plots. The observation in the control plot where the infection was higher may be a result of spread of propagules from plots inoculated in the previous year. A similar observation was recently reported in an open field situation by Bhoopander and Mukerji [25]. As expected, the enhancement of growth of inoculated plants was reflected in almost all the growth parameters measured in this experiment. This may be a direct effect of the

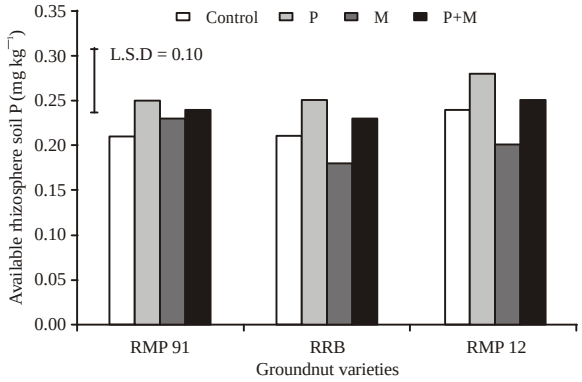

Fig. 3a: Effect of *Glomus mosseae* inoculation and phosphorus on available rhizosphere soil P in 2003 planting season

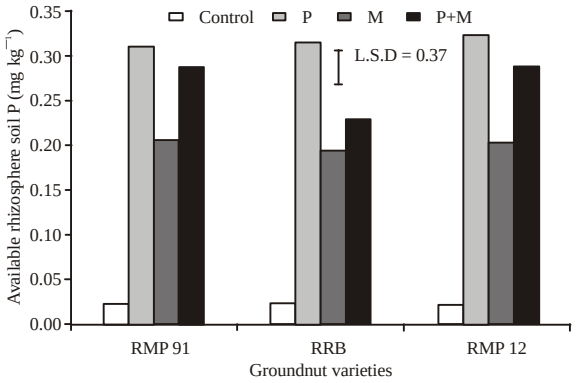

Fig. 3a: Effect of *Glomus mosseae* inoculation and phosphorus on available rhizosphere soil P in 2004 planting season

enhanced P uptake through the root as reported by Kothari *et al*. [26] and Li *et al*. [27] and may also have an inducing effect on other nutrients [28]. Similar increases in biomass production have been reported by Al-Karaki [29] and Al-Karaki *et al*. [30]. This study did not show any varietal difference all the parameters considered except the number of pods. The number of pods seemed higher in RRB but this did not translate to higher grain yield apparently due to lower shelling percentage recorded compared to the other two cultivars.

In this study, the grain yield in all the cultivars for the two seasons suggested equal effect of phosphorus fertilization at 54 kg haG¹ and inoculation with mycorrhizal and even compared to the plots with inoculation and phosphorus fertilization except in RMP_{12} where a marginal grain yield increase in plots fertilized and inoculated (P+M) was achieved. The increase may be linked with the phosphorus factor rather than the combined effects of phosphorus and inoculation as one may be tempted to belief. Earlier reports suggested suppression of infection and possible reduction in its effectiveness with increase availability of phosphorus in the soil [4, 31]. This claim is supported by the behavior of infections in the experiment where phosphorus fertilized plots had the lowest infection in all the groundnut cultivars in the first year and even much lower than the control in the second year. The inoculation of groundnut cultivars with the fungus contributed to its overall grain yield in the two planting seasons. The similarity in grain yield between all the treatments in RMP_{91} and RMP_{12} in the second year could be interpreted to mean that P fertilization at 54 kg haG¹ could produce the same effect on grain yield with inoculation. The enhanced yield in control plots in the second year could be attributed to the cross contamination of propagules from adjacent inoculated plots and resultant build-up of the fungal population and the subsequent increased root infection in the second year as observed by Bhoopander and Mukerji [24].

CONCLUSIONS

In this study, it is clear that groundnut can benefit from mycorrhizal symbiosis as a component of sustainable agriculture owing to its contribution to overall growth and grain yield. Also, for sustainable positive effect of inoculation, field inoculation may have to be annual until after the fungal population reaches a critical level. Inoculation of groundnut is not being canvassed as a substitute for phosphorus or any other soil nutrient but could allow the crop to put into use more of the soil nutrients per unit time. This will provide more time for replacement since nutrients are continuously removed during cropping and they need to be replaced. Further field studies in respect of other AM fungi for comparison, long term evaluation of field conditions as they affect the performance of mycorrhizal fungi and trials of mixture of inocula from different species of fungi deserve attention.

ACKNOWLEDGEMENTS

I thank Dr. J.O. Azeez of the Soil Science Department and S. Alade, of Plant Physiology and Crop Production Department for technical assistance in graphics and the field trial respectively. Many thanks to Prof. S.T.O. Lagoke of the Department of Plant Physiology and Crop Production for providing the groundnut seeds.

REFERENCES

1. Adelana, B.O. and V.O. Akintola, 1979. Effect of cultivation methods on the yield of groundnut in Western Nigeria. Nig. Agric. J., 16: 67-71.
2. Oyekami, P.O. and G.O. Agbaje, 1999. Performance of new improved groundnut varieties in the derived savanna area of south western Nigeria. Trop. Oil Seed J., 4: 35-40.
3. Daft, M.S. and A.A. El Giahmi, 1975. Effect of Glomus infection on tree legume. In Sanders, F.E., B. Mosse and P.B. Tinker, (Eds.). Endomycorrhizae. Academic Press New York, pp: 581-592.
4. Hayman, D.S., 1986. Mycorrhizal of nitrogen fixing legumes. MICREN J. Appl. Microbiol. Biotech., 2: 121-145.
5. Atayese, M.O., O.O. Awotoye, O. Osonubi and K. Mulongoy, 1993. Comparisons of the influence of vesicular-arbusular mycorrhiza on the productivity of hedgerow woody legumes and cassava at the top and the base of a hillslope under alley cropping systems. Biol. Fert. Soils, 16: 198-204.
6. Spencer, D.S.C. and K. Mulongoy, 1991. Development of technologies for productive and sustainable agriculture for the humid and sub humid tropics of Africa. In Amadoit Tidane B.A. and M. Ndoye (Ed.). The role of biology in resolving the food crisis in Africa. African Biol. Sciences Network (ABN) Dakar, Senegal, pp: 136-143.
7. Allen, C.B. and M.F. Allen, 1986. Water relations of xeric grasses in the field; interactions of mycorrhizal and competitions. New Phtol., 104: 559-571.
8. Reddel, P. and P. Warren, 1986. Inoculation of acacias with mycorrhizal fungi potential benefit. In Turnbull, J.W., (Ed.). Australian acacias in developing countries. Ac/Ar Proceeding No. 16 Brown Prior Anderson Press; Victoria, pp: 50-53.
9. Hussey, R.S. and R.W. Roncadori, 1982. Vesicular arbuscular mycorrhizal may limit nematode activity and improve plant growth. Plant Decease, 66: 9-14.
10. Miller, M.H., T.P. McGonigle and H.D. Addy, 1995. Functional ecology of vesicular arbuscular mycorrhizal as influential phosphate fertilization and tillage in an agricultural ecosystem. Crit. Rev. Biotech., 15: 241-255.
11. Mukeji, K.G., 1996. Concept in mycorrhizal research. Kluwer Academic Publishers, Netherlands.
12. Bryla, D.R. and R.T. Koide, 1990. Role of mycorrhizal infection in the growth and reproduction of wild accessions and twice cultwars of *Lycopersion esculentum* (mill) Oecologia, 84: 82-92.
13. Nelson, R.E. and L.E. Summers, 1982. Total carbon, organic carbon and organic matter. In: Page, A.L. (Ed.). Methods of Soil Analyses. Part 2 Agronomy No. 9 Madison Wis. USA.
14. Chapman, H.D., 1965. Cation exchange capacity. In Methods of soil analysis CA Black, (Ed.). Am. Soc. Agronomy No. 9 Madison Wis., pp: 891-901.
15. Bremner, J.M., 1965. Regular microkjedhal method for determination of total soil N: In Methods of soil analysis, Black, C.A. (Ed.). Am. Soc. Agron. No. 9 Madison Wis., pp: 1149-1176.
16. Murphy, J. and J.P. Rilley, 1962. A modified single solution method for the determination of phosphate in natural water. Anal. Chem. Anal., 27: 31-35.
17. Khalil, S., T.E. Loynachan and M.A. Tabatabai, 1994. Mycorrhizal deficiency and nutrient uptake by improved and unimproved corn and soybean cultivars. Agron. J., 86: 949-958.
18. Nichporovich, A.A., 1983. Determination of leaf area of soybean plants. In: Methodology of field agrotechnical experiments on soybeans and their observations. Baramov, B.F., A.I. Lebedovsky and I.N. Terentiev (Eds.). Vseoguznar Sel'skhozyaistvennykh Nawk Im. V.I. Lenina. Krasnodar, pp: 7.
19. Giovanetti, M. and B. Mosse, 1980. An evaluation of technique for measuring vasicular arbuscular mycorrhizal infection in roots. New Phtol., 84: 489-500.
20. Simpson, D. and M.J. Daft, 1990. Effect of *Glomus clarum* and water stresss on growth and nitrogen-fixation in two genotypes of groundnut. Agric. Ecosys. Environ., 35: 47-54.
21. Harley, J.L. and S.E. Smith, 1983. Mycorrhizal symbiosis. Academic Press, New York, pp: 483.
22. Sieverding, E., 1991. Vesicular-arbuscular mycorrhizae management in tropical agrosystems. Deutsche Gesellschaft für Tecnische Zusammenarbet (GTZ) Eschborn, Germany.
23. Hawkins, H.J. and E. George, 1999. Effect of plant nitrogen status on the contribution of arbuscular mycorrhizal hyphae to plant nitrogen uptake. Physiol. Plant., 105: 694-700.

24. Osonubi, O., M.O. Atayese and K. Mulongoy, 1995. The effect of vesicular arbuscular mycorrhizal inoculation on nutrient uptake and yield of alley cropped cassava in a degraded Alfisol of south western Nigeria. Biol. Fert. Soils, 20: 70-76.
25. Bhoopander, G. and K.G. Mukerji, 2004. Mycorrhizal inoculant alleviates salt stress in Sesbania aegyptica and Sesbania grandiflora under field conditions: Evidence for reduced sodium and improved magnesium uptake. Mycorrhiza, 14: 307-312.
26. Kothari, S.K., H. Marschner and V. Romheld, 1991. Contribution of the VA mycorrhizal hyphae and aquistion of phosphorus and zinc by maize grown in calcareous soil. Plant and Soil, 131: 177-185.
27. Li, X., E. Geoge and H. Marschner, 1991. Phosphorus depletion and pH decrease at the root-soil and hyphae-soil interfaces of VA mycorrhizal white clover fertilized with ammonium. New Phytol., 119: 397-104.
28. Bowen, G.D., 1985. Microrganisms and tree growth. In: Landsberg, J.J. and W. Pearsons, (Eds.). Research for Forestmanagement. CSIRO, Mebourne, pp: 180-201.
29. Al-Karaki, G.N., 2000. Growth of mycorrhizal tomato and mineral acquisition under salt stress. Mycorrhiza, 10: 51-54.
30. Al-Karaki, G.N., R. Hammad and M. Rusan, 2001. Response of two cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. Mycorrhiza, 11: 43-47.
31. El-Tohamy, W., W.H. Schnitzler, U. El-Behairy and M.S. El-Beltagy, 1999. Effect of VA mycorrhiza on improving drought and chilling tolerance of beans plants (*Phaseolus vulgaris* L.) Angewandte Botanik, 73: 178-183.

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 24-32, 2007
ISSN 1818-6769

Water Saving in Crop Production in the Humid and Semi-Arid Tropical Regions by Optimal Compost Applications

[1,2]*Molengar Ngoundo*, [3]*Kan Chun-E*, [4]*Chang Yu-Chuan*, [5]*Shiow-Long Tsai and* [6]*I. Tsou*

[1]Counterpart Chief of Taiwan Technical Mission,
National Office of Rural Development (ONDR), Ministry of Agriculture, Republic of Chad
[2]Department of Tropical Agriculture and International Cooperation,
National Pingtung University of Science and Technology, Pingtung, Taiwan, ROC
[3]National Taiwan University, Republic of China, ROC,
[4]National Leader University, Republic of China, ROC
[5]Department of Plant Industry, [6]Department of Civil Engineering,
National Pingtung University of Science and Technology, Pingtung, Taiwan, ROC

Abstract: Water saving technology interests crop producers in both the humid and semi-arid regions of the tropics. Although water saving does not always match with high yield, the surplus of water can be used effectively and efficiently to produce various crops. The amount of irrigation water used could substantially be reduced by the application of compost. The target environments are lowland areas with water shortage and favorable upland with access to increase soil available water capacity. The objective of this study was to assess the number of irrigation applications and water savings through the optimum rate of compost application. Results show that in both tropical humid and semi-arid regions, compost application on some "thirstiest" and cash crops is a reliable means for water savings. The net irrigation depths substantially raised from the soil treated with chemical fertilizers to the compost treatments and as a consequence, reduced the number of irrigation applications by 16-12% in amended-soil. The difference in the number of irrigation applications ranged from 1 to 2 and 1 to 5 applications in tropical humid and semi-arid regions, respectively. With rainfall, supplemental irrigation was only necessary for rice and onion in tropical humid regions, while in semi-arid regions, irrigation constituted the main source of crop water supply. The potential water saved was estimated at 154.74, 5.40% and 34.94, 14.53% of the irrigation water need with and without rainfall in tropical humid and semi-arid regions, respectively and accounted for about 1.62% in average of the total Lake-Chad annual inflow. The amount of water saved could be used to double the growing areas of some cash crops and to recharge the underground water for water resource conservation and environmental restoration, supply to Lake-Chad or allocate to other water use sectors.

Key words: Compost % potential water saving % tropical humid % semi-arid regions % environment restoration

INTRODUCTION

Water saving technology should not only interest the arid and semi-arid regions, but also the tropical humid areas because of the uneven distribution of precipitation. Kuo *et al*. [1] in assessing the crop water requirements in the Taiwan ChiaNan irrigation district, emphasized that the unevenly distribution of rainfall is not always optimal for the growing seasons of various crops. Although water saving does not always match with high yield, the surplus water could be effectively and efficiently used to produce various other crops. Bouman and Tuong [2] suggested that paddy rice irrigation water input can be reduced and water productivity increased by introducing periods of nonsubmerged conditions of several days throughout the growing season. In subtropical area of China, systems of alternate submergence-nonsubmergence on rice production have been reported to maintain or even increase yield [3]. However, experimental evidence is still scarcely reported in the literature and the hydrological and environmental conditions under which these systems were practiced are not well known [4]. In lowland field and irrigated upland crops, the irrigation water use could substantially be reduced by

Corresponding Author: Dr. Molengar Ngoundo, Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung, Taiwan, R.O.C. 1. Hsueh Fu rd, 91201

the application of compost. The target environments are irrigated lowland with water shortage and favorable upland with access to increase soil available water capacity. In practice, irrigation can be applied to bring the soil water capacity up to field capacity once a lower threshold has been reached. For upland crops such as wheat and maize, this threshold is usually the soil water content halfway between field capacity and wilting point [5], but for upland rice, the optimum threshold for re-irrigation still needs to be determined [4]. This water content between field capacity and wilting point is defined as the available water capacity. Many dairy farmers apply organic manure predominately for fertilizing the soil and manure application could improve the soil properties, thereby improving the N balance. Organic manure has been found to be a reliable index of crop productivity in semi-arid regions because it positively affects soil water holding capacity [6, 7]. The beneficial effect of compost on crop growth is well known, however, there has been no report emphasizing its effect on crop irrigation water savings. Therefore, study on compost application on clay soil in both humid and semi-arid regions and its effect on irrigation water savings could contribute not only to lower the irrigation water inputs, but also to regenerate the already degraded environment. The objective of this study was to assess the number of irrigation applications and potential water saving through the optimum rate of compost application.

MATERIALS AND METHODS

Climate data: The reference evapotranspiration (ET_0), crop evapotranspiration (ET_{crop}) and Effective Rainfall (ER) were determined by computing 15-year average climate data from National Pingtung University of Science and Technology (NPUST) and Central Weather Bureau (CWB) of Taiwan located at 22° 39'N and 120°36'E and the meteorological station of NDjamena in the Republic of Chad situated at 15°00'N and 19°00'E, respectively, using the CROPWAT software [8]. The monthly mean maximum and minimum temperatures varied from 25.1°C in January to 33.2°C in July and 14.2°C in January to 24.2°C in July, respectively in Taiwan while it ranges from 32.2°C in January to 38.1°C in June and 14.7°C in January to 26.7°C in May, respectively in the Republic of Chad. Moreover, the monthly relative humidity ranges from 77.5% in March to 85.0% in August and 20.0% in March to 78.0% in August in Taiwan and Chad, respectively. The monthly mean solar radiation varied from 10.9 MJ m^{-2} d^{-1} in December to 16.4 MJ m^{-2} d^{-1} in June and 20.1 MJ m^{-2} d^{-1} in December to 23.9 MJ m^{-2} d^{-1} in May in Taiwan and Chad, respectively, while the wind speed and sunshine varied from 98.0 km d^{-1} in November to 292.0 km d^{-1} in May and 3.4 h in May to 4.6 h in January in Taiwan and 224.6 km d^{-1} in August to 449.3 km d^{-1} in March and 7.0 h in July to 10.1 h in December in Chad.

As a consequence the reference evapotranspiration ranges from 2.3 mm d^{-1} in December to 4.4 mm d^{-1} in April and 4.8 mm d^{-1} in August to 11.6 mm d^{-1} in February in Taiwan and Chad, respectively. The effective rainfall on the other hand ranges from 15.1 mm month^{-1} in December to 188.9 mm month^{-1} in August and 0.0 mm month^{-1} in January, February, March and December to 132.7 mm month^{-1} in August in Taiwan and Chad, respectively (Table 1).

Soil texture and available water capacity: The simplified hydrometer method described in the Particles Size Distribution [9] was used to determine the texture of four types of soil (3 from Taiwan and 1 from Chad). The textures of the soils were clay, sandy-loam and loamy-sand in Taiwan and clay in Chad with the specific gravity and available water capacity plotted in Table 2.

The virgin and compost mixed with sandy-loam and loamy-sand soils exhibited low available water capacity (Table 2 and Fig. 1). The target is to save water in lowland area, therefore, the Taiwan and Chad clay soils with high available water capacity were selected to investigate water saving. Clay soils were mixed with chemical fertilizer N-P-K (120:70:70) kg ha^{-1} as a control (usually utilized by Taiwanese farmers) and compost 5, 10, 5, 20, 30, 45 t ha^{-1}. The mean specific gravities (dry weight basis) and corresponding Available Water Capacity (AWC) are showed in Table 3.

The net irrigation depths (d) were determined using the soils available water capacity, the specific gravity and the root depth, defined as:

$$d(mm) = \alpha(AWC * As * D)\ [10] \quad (1)$$

Where AWC is the available water capacity (%) [11];

" = 1 for paddy rice, ¾ for medium rooting cropping and ½ for shallow rooting cropping;

As is the soil specific gravity;

D: The root depth (mm).

The irrigation intervals (INT) and the number of irrigation applications (NIA) were calculated as followed:

Table 1: 15-year average evapotranspiration and effective rainfall of NPUST/CWB and NDjamena stations (1990-2004) month

	Taiwan (NPUST/CWB)			Chad (Ndjamena)		
	ET_0 (mm day^{-1})	Rainfall (mm month^{-1})	ER[(a)] (mm month^{-1})	ET_0 (mm day^{-1})	Rainfall (mm month^{-1})	ER[(a)] (mm month^{-1})
January	2.4	28.2	26.9	7.8	0.0	0.0
February	2.8	28.8	27.5	9.5	0.0	0.0
March	3.4	36.6	34.5	11.6	0.0	0.0
April	3.7	96.4	81.5	10.9	8.4	8.3
May	4.4	244.9	148.9	10.1	28.5	27.2
June	4.1	389.0	163.9	8.2	49.8	45.8
July	4.0	464.9	171.5	6.4	171.6	124.5
August	3.7	639.3	188.9	4.8	191.1	132.7
September	3.4	326.5	157.6	5.5	99.7	83.8
October	3.0	81.1	70.6	6.9	31.3	29.7
November	2.6	15.6	15.2	8.4	2.6	2.6
December	2.3	15.5	15.1	7.7	0.0	0.0
Total (mm year^{-1})	3.3	2366.8	1102.1	8.1	583.0	454.6

[(a)]Effective rainfall calculated using the United States Soil Conservation Service (USSCS) formulas: Effective R. = (125-0.2 * Total R.)* Total R./125... (Total R.<250 mm/month), Effective R. = 0.1 * Total R.-125 (Total R. > 250 mm/month)

Table 2: Texture of soils used for determining the effect of compost application on soil available water capacity (weight basis)

Soil code	Sand (%)	Clay (%)	Silt (%)	Specificgravity	AWC (%)	Textural class
TCS	26.83[c*]	46.28[b]	26.89[a]	1.30[d]	26.2[a]	Clay
TSLS	62.30[b]	14.67[c]	23.30[a]	1.40[c]	24.9[c]	Sandy loam
TLSS	76.19[a]	7.65[d]	16.16[b]	1.45[b]	17.5[d]	Loamy sand
CCS	22.47[c]	60.11[a]	17.42[b]	1.55[a]	25.3[b]	Clay

*Means with the same letter within column are not significantly different at 5% level (Duncan MRT). TCS: Taiwan clay soil, TLSS: Taiwan loamy-sand soil, TSLS: Taiwan sandy-loam soil, CCS: Chad clay soil

Table 3: Specific gravity and available capacity of some amended Taiwan and Chad clay soils

	Taiwan		Chad	
Treatments	Specific gravity	AWC (%)	Specific gravity	AWC (%)
120:70:70	1.30	25.80	1.51	25.00
C5*	1.28	27.50	1.51	26.50
C10	1.27	28.40	1.50	27.40
C15	1.26	28.00	1.49	27.20
C20	1.24	28.50	1.48	25.40
C30	1.22	28.20	1.47	26.00
C45	1.20	27.20	1.46	25.00

* C5, C10, C15, C20, C30, C45 Compost 5, 10, 15, 20, 30 and 45 t ha^{-1} treatments

$$INT = \frac{d}{(ETcrop - ER)} GD \quad (2)$$

Where; (d) is the net irrigation depth (mm); ER is the seasonal effective rainfall (mm); ET_{crop} is the crop water need (mm); GD is the crop growth duration (days); and the number of irrigation applications (NIA):

$$NIA = \frac{GD}{INT} \quad (3)$$

Rice, sugarcane, wheat, three "thirstiest" crops [12] and tomato, onion, cabbage, the most irrigated cash crops were selected for this study in tropical humid (Taiwan) and semi-arid (Chad), two different climatic regions.

Crops coefficients were monthly determined from that of different growth stages defined by Doorenbos and Pruitt [5]. Analysis of variances and graphs were carried out using SAS V.8 and SigmaPlot 2001 softwares.

RESULTS AND DISCUSSION

Effect of compost application on the soil available water capacity: The available water capacity of Taiwan and Chad clay soils was estimated based on the compost application (Fig. 1) and reflected about 91% and 78% the variation of compost application (Eq. 4 and 5).

The available water capacity increased to reach the maximum and then declined; hence there exists a point at

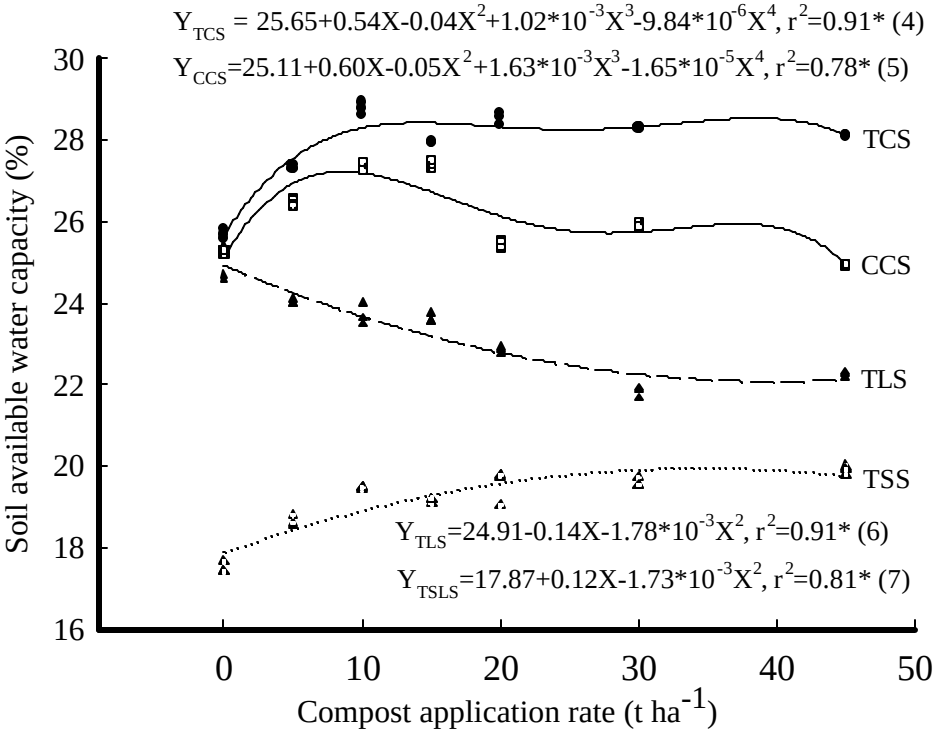

Fig. 1: Soil available water capacity in response to compost application on Taiwan and Chad soils

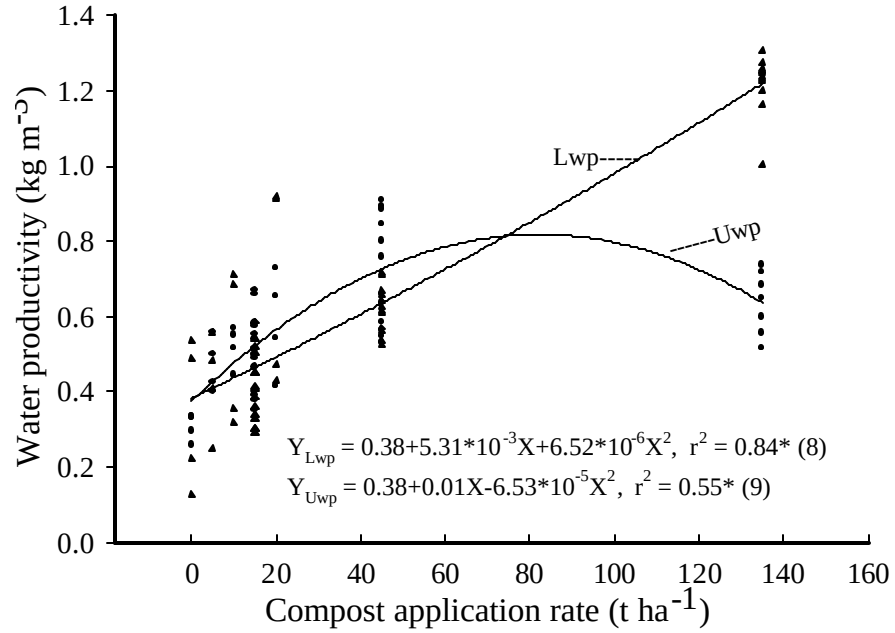

Fig. 2: Water productivity in response to compost application on clayey soils

Table 4: Monthly crop coefficients (K_c)

	Months												
Crops	Jan	Feb	March	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan
Rice1*							1.10	1.10	1.15	1.05	1.00		
Rice2		1.10	1.10	1.25	1.00								
Wheat						0.35	0.75	1.15	0.45				
Sugarcane		0.45	0.60	0.85	1.00	1.20	1.30	1.30	1.30	1.30	1.05	0.75	
Tomato		0.45	0.70	0.95	1.15	0.85							
Onion				0.65	0.95	1.05	1.00	0.85					
Cabbage									0.45	0.70	0.75	1.05	0.90

*1 and 2: Fall and spring rice

Table 5: Crop evapotranspiration (mm) and effective rainfall (mm) in the growth period

	Growth*		Taiwan (NPUST/CWB) stations		Chad (NDjamena) station	
Crops	Period	Duration	ET_{Crop}	ER	ET_{Crop}	ER
Rice1	Aug-Dec	130	918.70	447.50	1440.40	248.80
Rice2	Feb-May	120	952.90	292.40	1886.80	35.50
Wheat	June-Sept	120	303.60	681.60	469.30	386.80
Sugarcane	Feb-Dec	320	1112.40	1102.10	2452.80	454.60
Tomato	Feb-June	150	467.90	754.70	774.30	338.50
Onion	Apr-Aug	150	536.20	456.30	1071.70	81.30
Cabbage	Sept-Jan	120	264.00	285.40	816.52	116.10

*Data from FAO paper No. 24 [5], ER: Effective Rainfall, 1 and 2 fall and spring rice

which the derivative of those functions equals to zero. The optimum compost application rates and the corresponding available water capacity at those maxima were determined at 10.21 t ha^{-1}, 28.0% and 10.64 t ha^{-1}, 27.5% respectively, using the trial error Newton method. The values were used to assess the level of water productivity and the crop net irrigation depth. From the above figure, the compost application on clay soil significantly improved the available water capacity.

Water productivity as affected by the application of compost: Rice productivity was determined from the data of two growing season-experiments in a control green house conditions. *Oriza Sativa* L. variety Taichung No.10 was grown under lowland conditions using chemical fertilizer N-P-K (120:70:70) kg ha^{-1} and 5, 10, 15, 20, 45 and 135 t ha^{-1} of compost. The water productivity obtained in those treatments ranges from 0.38 to 1.22 kg m^{-3} and 0.38 to 0.70 kg m^{-3} under lowland and upland conditions, respectively.

The change in water productivity reflected about 84% and 55% the variation of compost application in lowland and upland crops respectively as defined in the equations (8) and (9) in Fig. 2.

From the compost application vs. water productivity equations, it can be observed that water productivity were 0.43 and 0.48 kg m^{-3} and 0.44 and 0.48 kg m^{-3} under lowland and upland conditions using the optimum compost rates (10.21 t ha^{-1} and 10.64 t ha^{-1}) in Taiwan and Chad, respectively. The values of water productivity obtained through the equations were in the range reported by some previous studies. The rice water productivity in 1995 ranged from 0.15 to 0.60 kg m^{-3} and the sustainable increases in water productivity can only be achieved through integrated farm-resources management [13, 14]. Tuong and Bouman [15] summarized a range of water productivity from 0.4 to 1.1 kg m^{-3} for rice in farmer fields and irrigation systems of Northwest India, therefore the subsequent values of available water capacity generated by the optimum compost rates can be efficiently used in accessing crop water need.

Irrigation water need: The long-term meteorological data from 1990 to 2004 of the NPUST-CWB in Taiwan and Ndjamena-Chad meteorological stations were used to calculate the reference evapotranspiration. The crop water need was calculated on a monthly basis using:

$$ET_{crop} = ET_0 * K_c \quad [10]$$

Where; ET_{crop}: is the crop evapotranspiration, ET_0: the reference evapotranspiration, K_c is the crop coefficients.

Table 6: Net irrigation depths, irrigation intervals, number of irrigation applications and irrigation water saving determined on chemical fertilizer and compost treatments in Taiwan and Chad

		Soil amended with chemical fertilizer			Soil amended with compost			Water savings
Regions	Crops	d (mm)	INT (days)	NIA (No.)	d (mm)	INT (days)	NIA (No.)	(mm)
Taiwan	Rice1	134	18	8	142	20	7	142
(Pingtung)	Rice2	134	16	8	142	17	7	142
	Wheat	100	39	4	107	42	3	107
	Sugarcane	150	43	8	160	46	7	160
	Tomato	67	21	8	71	22	7	71
	Onion	33	9	17	36	10	15	72
	Cabbage	33	15	8	36	16	7	36
Chad	Rice1	151	13	10	165	14	9	165
(NDjamena)	Rice2	151	9	14	165	10	12	330
	Wheat	113	28	5	124	31	4	124
	Sugarcane	170	22	15	186	24	14	186
	Tomato	76	14	11	83	15	10	83
	Onion	38	5	30	41	6	25	205
	Cabbage	38	5	24	41	6	20	164

The crop coefficient K_c (Table 4) based on the growth stages [16] were calculated on a monthly basis. The Table 5 shows the crop water need, growth duration and the effective rainfall during the growth of crops. The crop water need during the growth period varied from 264.0 mm on cabbage to 1112.4 mm on sugarcane in Taiwan, while it ranges from 469.3 mm on wheat to 2452.8 mm on sugarcane in Chad. For lowland rice, the water for soil saturation (200 mm), percolation (180 mm month^{-1}) and water layer (100 mm) [5] were added to the crop evapotranspiration. The difference of water need between those two regions was attributed to the higher evapotranspiration observed in NDjamena station. The effective rainfall varied from 285.4 mm in the period where the cabbage was grown to 1102.1 mm on sugarcane in Taiwan and 35.5 mm on rice (Feb-May) to 454.6 mm on sugarcane (Feb-Dec) in Chad. The large difference between precipitation and ET_{crop} means that irrigation water resources are the most important limiting factor for sustainable crop production in this area.

The irrigation water need of rice and sugarcane were similar to the one obtained by Kuo *et al*. [1]. In the Republic of Chad, irrigation water need was higher for rice, wheat, sugarcane, tomato, onion and cabbage, respectively (Table 5). Those data were in the ranges given by the Rural Development Integrated Project of Chari-Logone Basin (Chad-Cameroon) in the feasibility study [17] and FAO No. 24 [5].

Irrigation water depth, number of irrigation applications and water saving: The rooting depths of rice, wheat and tomato were estimated at 40 cm that of sugarcane, onion and cabbage at 60 cm and 20 cm respectively. Table 6 shows the net irrigation depths, the irrigation intervals and the number of irrigation applications in chemical fertilizer and the optimum compost treatments. The net irrigation depths were 33 mm on cabbage and onion, 67 mm on tomato, 100 mm on wheat, 150 mm on sugarcane and 134 mm on rice in Taiwan while it was 38, 76, 113, 170 and 151 mm in chemical fertilizer treatments in Chad. In compost treatments, the net irrigation depths were 36, 71, 107, 160 and 142 mm in Taiwan and 41, 83, 124, 186 and 165 mm in the Chad clay soil. Irrigation intervals on the other hand, varied from 9 days on onion to 43 days on sugarcane in Taiwan and 5 days on onion to 28 days on wheat in Chad in the chemical fertilizer treatments. In the compost treatments, they range from 10 days on onion to 46 days on sugarcane in Taiwan and 6 days on onion to 31 days on wheat in Chad. Consequently, the number of irrigation applications was substantially low in both tropical humid and semi-arid regions. The difference in the number of irrigation applications varied from 1 to 2 and 1 to 5 applications in Taiwan and Chad, respectively As a result, the water savings during the growing period ranges from 36 to 160 mm in Taiwan and 83 to 330 mm in Chad.

Considering the contribution of the rainfall during the growing period, wheat, sugarcane, tomato and cabbage do not need any irrigation in Taiwan, while supplemental irrigation ranged from 1 to 25 applications were necessary for completing plant growth in Chad under amended soil. Nevertheless, potential huge

Table 7: Irrigation intervals, number of irrigation applications and irrigation water saving determination taking into account the amount of precipitation during the growing period in Taiwan and Chad

Region	Crop	Supplemental irrigation water need (mm)	Soil amended with compost under rainfall			Water savings in compost compared to that of chemical fertilizer treatments (mm)
			d (mm)	INT (days)	NIA (No.)	
Taiwan	Rice1	471.21	142	39	4	568
(Pingtung)	Rice2	660.46	142	25	5	426
	Wheat	-378.04	107	0	0	428
	Sugarcane	10.32	160	0	0	1280
	Tomato	-286.77	71	0	0	568
	Onion	79.86	36	66	3	504
	Cabbage	-21.38	36	0	0	288
Chad	Rice1	1191.59	165	18	8	330
(NDjamena)	Rice2	1851.34	165	10	12	330
	Wheat	82.54	124	179	1	496
	Sugarcane	1998.16	186	29	11	744
	Tomato	692.95	83	28	6	415
	Onion	733.16	41	6	25	205
	Cabbage	700.42	41	7	18	246

No irrigation is needed showing by the excess of the amount of rainfall indicated with (-)

Table 8: Amended soil water saving in fall and spring cropping seasons in Taiwan and Chad

Regions	Crops	Area (ha)	Without rainfall			With rainfall	
			Irrigation water need ($10^6 m^3$)	Water saved (10^6 m^3)	Percent water saved (%)	Water saved (10^6 m^3)	Percent water saved (%)
Taiwan	Rice1	135314	1243.14	192.15	15.46	768.58	61.83
	Rice2	161700	1540.77	229.61	14.90	688.84	44.71
	Wheat	12343	37.47	13.21	35.25	52.83	140.99
	Sugarcane	777	8.64	1.24	14.39	9.95	115.11
	Tomato	5043	23.60	3.58	15.17	28.64	121.37
	Onion	834	4.47	0.60	13.43	4.20	94.03
	Cabbage	8084	21.34	2.91	13.64	23.28	109.10
	Total	324095	2879.43	443.30	15.40	1576.33	54.74
Chad	Rice1	5500	79.22	9.08	11.46	18.15	22.91
	Rice2	4500	84.91	14.85	17.49	14.85	17.49
	Wheat	9000	42.24	11.16	26.42	44.64	105.68
	Sugarcane	3754	92.08	6.98	7.58	27.93	30.33
	Tomato	2000	15.49	1.66	10.72	8.30	53.58
	Onion	2000	21.43	4.10	19.13	4.10	19.13
	Cabbage	2000	16.33	3.28	20.09	4.92	30.13
	Total	28754	351.70	51.11	14.53	122.89	34.94

amounts of water were saved compared to the water use in chemical fertilizer treatments without rainfall. They varied from 288 to 1280 mm in Taiwan and 205 to 744 mm in Chad (Table 7). The supplemental irrigation observed in all crops in the Republic of Chad indicated that the irrigation was the main source of crop water supply in the scarce precipitation regions.

Irrigation water saved in tropical humid (Taiwan) and semi-arid (Chad) regions: a simulated case study: In the Republic of Chad, the agricultural sector is the largest water consumer [18]. Out of the total of 30273 ha of the irrigated crop areas, 3754 ha of sugarcane are irrigated by sprinklers and the remaining 26519 ha by gravity. The main "thirstiest" crops (rice, wheat, sugarcane) and cash crops (tomato, onion, cabbage) irrigated areas are shown in Table 8. There are 10000, 9000, 3754 ha of rice, wheat and sugarcane, respectively and 2000 ha for each cash crop including tomato, onion and cabbage. A total of 28754 ha were irrigated with irrigation water need of about $351.70*10^6$ m^3, ranging from $15.49*10^6$ m^3 on tomato to $92.08*10^6$ m^3 on sugarcane. Total water savings were

estimated at about $51.11*10^6$ m^3 representing 14.53% of the total water need in the conditions without rainfall. Including rainfall, the total water saved was $122.89*10^6$ m^3 or 34.94% of the total water consumption. In both tropical humid and semi-arid regions, the use of compost on the "thirstiest" as well as on cash crops is a reliable means for water saving. These amounts of water saved can be used to double the area of wheat, tomato, onion and cabbage, to recharge the underground water, or to supply Lake-Chad as suggested by Odada *et al*. [19] who stipulated that, due to the lowering level or drying of the Lake Chad and of the reduced inflows of its main rivers, every new irrigation development must be studied very carefully for a sustainable agriculture. Extended to the whole Lake-Chad Basin Commission actually irrigated area estimated at about 115,000 ha [19], potential water saving using the compost application technology contributed in average to about 1.62% of the total annual water inflow (43 km^3 $year^{-1}$) of lake-Chad [18]; this technology could substantially contribute to the restoration of the environment by recharging Lake-Chad.

In Taiwan, the tropical humid area, a total of 324095 ha of those selected crops were irrigated in 2004 [20] with a total water need of $2879.43*10^6$ m^3. The potential annual water saving using this new technology was estimated at about $443.30*10^6$ m^3, which represents 15.40% of the total water need without rainfall. Including rainfall, the irrigation water saving was about $1576.33*10^6$ m^3 or 54.74% of the total water need.

Individual potential water savings over 100% could be explained as the improvement of the available water capacity under the effect of compost application. This amount of water saved can be used in agriculture to supplement the deficit irrigation caused by the unevenly rainfall distribution in upland, or in the other water use sectors.

CONCLUSIONS

In both tropical humid and semi-arid regions, compost application on some "thirstiest" and cash crops, is a reliable means for water savings. The net irrigation depths substantially raised in the compost amended-soil compared to chemical fertilizer treatments and as a consequence, lowered the number of irrigation applications in compost treatments. The difference in the number of irrigation applications ranged from 1 application on cabbage to 2 applications on sugarcane and 1 to 5 applications on wheat and onion in tropical humid and semi-arid regions, respectively. With the contribution of rainfall, supplemental irrigation was only necessary for rice and cabbage in tropical humid region, while in semi-arid regions, irrigation constitutes the main source of crop water supply. The total water saving was estimated at 14.53, 34.94% and 15.40, 54.74% of the total irrigation water need without and with rainfall in tropical semi-arid and humid regions, respectively. The amount of water saved which represented 1.62% on average of the total Lake-Chad annual inflow, can be used to double the growing areas of some upland crops, to recharge the underground water for water resource conservation and environmental restoration or to supply to Lake-Chad. In tropical humid regions, this water saved could be used as a supplemental irrigation or allocate to the other water use sectors. In both tropical humid and semi-arid regions, the use of compost on "thirstiest" as well as on cash crops is a reliable means for water saving. Although water saving on sugarcane is as high as that of grain crops, the investigation of crop sugar content constitutes a precondition for final conclusion.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Babou Job for his valuable comments and suggestions on this manuscript.

REFERENCES

1. Kuo, S.F, S.S. Ho and C.W. Liu, 2005. Estimation irrigation water requirements with derived crop coefficients for upland and paddy crops in ChiaNan Irrigation Association, Taiwan. Agric Water Manag. vailable online at www.Sciencedirect.com, 82: 433-451.
2. Bouman, B.A.M. and T.P. Tuong, 2001. Field water management to save water and increase its productivity in irrigated lowland rice. Agric. Water Manag., 49: 11-30.
3. Li, Y.H., 2001. Research and practice of water saving irrigation for rice in China. In Water-saving irrigation for rice. Barker, R., R. Loeve, Y.H. Li, T.P.Tuong (Eds.). Proceedings of an International Workshop 23-25 March, 2001, at Wuhan, China, pp: 135-144.
4. Belder, P., B.A.M. Bouman, R. Cabangon, L. Guoan, E.J.P. Quilang, L. Yuanhua, J.H.J. Spiertz and T.P. Tuong, 2004. Effect of water-saving irrigation on rice yield and water use in typical lowland conditions in Asia. Agric. Water Manag., 65: 193-210.

5. Doorenbos, J. and W.O. Pruitt, 1984. Guidelines for predicting crop water requirements. FAO Irrigation and Drainage Paper 24, Food and Agriculture organization, Rome, pp: 144.
6. McDaniel, P.A. and L.C. Munn, 1985. Effect of temperature on organic carbon-texture relationships in Mollisols and Aridisols. Soil Sci. Soc. Am. J., 49: 1486-1489.
7. Buschiazzo, D.E., A.R. Quiroga and K. Stahr, 1991. Patterns of organic matter accumulation in soils of the Semiarid Argentinian pampas. Z. Pflanzenernähr. Bodenk, 154: 437-441.
8. FAO, 1992. CROPWAT - A Computer Program for Irrigation Planning and Management. FAO Irrigation and Drainage Paper No. 46. Food and Agriculture Organization, Rome.
9. Sheldrick, B.H. and C. Wang, 1993. Particle Size Distribution. Agriculture Canada, Ottawa, Ontario. In: Soil Sampling and Method of Analysis. Carter, M.R. Ed. Can. Soc. Sci., pp: 499-511.
10. Tsing, S. and J.Y. Zhen, 1980. On-farm irrigation water use. In: Manual for Upland Crop Irrigation, pp: 139-170, (In Chinese).
11. Cassel, D.K. and D.R. Nielson, 1986. Field capacity and available water capacity. In Methods of Soil Analysis. Part.1, Klute A., (Ed.), Madison: Am. Soc. Agron., pp: 901-926.
12. Martin, C., 2003. Agricultural Water Use and River Basin Conservation. A summary report compiled and edited by Tim Davis. D.J., Environmental. UK. Published by World Wide Fund for nature, Gland, Switzerland.
13. Kijne, J., R. Barker and D. Molden, 2003. Water productivity in agriculture: Limits and opportunities for improvement. Comprehensive assessment of Water Management in Agriculture, Series No. 1, CABI Press, Wallingford, UK., pp: 352.
14. Oweis, T.Y. and A.Y. Hachum, 2003. Water Productivity in Agriculture: Limits and Opportunities for Improvements. Kijne, J.W., R. Barker and D. Molden (Eds.), pp: 179-198.
15. Tuong, T.P. and B.A.M. Bouman, 2003. Rice production in water-scarce environments. In Kijne, J., R. Barker, D. Molden, (Eds.). Water Productivity in Agriculture: Limits and opportunities for improvements. Comprehensive assessment of Water Management in Agriculture, Series No.1, CABI Press, Wallingford, UK., pp: 167.
16. Brower, C., M. Heibloem and J.V. Dijk, 1986. Irrigation Water Management. Training manual No. 3. FAO, Via delle Terme di Caracalla, 00100 Rome, Italy.
17. Hydroconsult International, Dakar, Sénégal and Turkpak International (PVT) Limited, Lahore, Pakistan, 2004. Projet de Développement Intégré du Chari-Logone (Cameroun-Chad). Etude de Faisabilité., Annexures, 2: E1-E11.
18. AQUASTAT, 2005. L'irrigation en Afrique en chiffres-Enquête. Système d'information de la FAO sur l'eau et l'agriculture.
19. Odada, E.O., L. Oyebande and J.A. Oguntola, 2005. Lake Chad, Experience and Lessons Learned Brief. Water resource unit, Lake Chad Basin Commision, Ndjamena, Chad.
20. Agricultural Statistics Yearbook, 2004. Agriculture and Food Agency, COA Executive Yuan, pp: 23-73. http://www.fao.org/ag/agl/aglw/aquastat/countries/chad/indexfra.stm

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 33-38, 2007
ISSN 1818-6769

Response of Kalmegh to an Arbuscular Mycorrhizal Fungus and a Plant Growth Promoting Rhizomicroorganism at Two Levels of Phosphorus Fertilizer

J. Arpana and D.J. Bagyaraj

Department of Agricultural Microbiology,
University of Agricultural Sciences, G.K.V.K. Campus, Bangalore 560065, India

Abstract: A field investigation was conducted to know the influence of inoculation with the Arbuscular Mycorrhizal (AM) fungus *Glomus mosseae* and the Plant Growth Promoting Rhizomicroorganism (PGPR) *Trichoderma harzianum* singly and in combination on growth and yield of kalmegh (*Andrographis paniculata*) at two levels of P fertilizer application i.e. at the recommended level and 75% of the recommended level. The plant height, plant spread, number of branches per plant, number of leaves per plant, leaf area, plant dry matter, plant P content and andrographolide (alkaloid of pharmaceutical importance) concentration were significantly higher in plants inoculated with both the organisms, at both the levels of P as compared to uninoculated plants. Inoculation with both the organisms also reduced significantly days for 50% flowering. Higher root colonization and spore numbers in the root zone soil were also observed when both the organisms were inoculated, at both the levels of P. This clearly brings out that inoculation with *G. mosseae* + *T. harzianum* not only improved growth, biomass yield, P nutrition and andrographolide concentration of kalmegh but also helped in saving 25% of P fertilizer application.

Key words: *Andrographis paniculata* % Arbuscular mycorrhiza % *Glomus mosseae* % kalmegh % *Trichoderma harzianum*

INTRODUCTION

Kalmegh (*Andrographis paniculata* Nees.) belonging to the family Acanthaceae is one of the medicinal plants that is recommended for cultivation in India, as there is a great demand for the plant by the pharmaceutical industries mainly for export. It is a source of several diterpenoids of which andrographolide (alkaloid) is important. The drug is used for treating general debility, dyspepsia, chronic malaria, jaundice and dysentery. Some scientists have observed that andrographolide has the potential to be included in the cocktail vaccine against AIDS by virtue of its antagonistic property with HIV II virus [1]. It has been already used for treating cancer as it promotes cell differentiation in tumour cells [2]. Though leaves contain maximum andrographolide, the entire plant is used for extracting the active ingredient.

Currently, the emphasis is on sustainable agriculture, which uses less of chemical inputs like fertilizers and pesticides having adverse effect on soil health, fertility and environment. Thus microbial inoculants play an important role in sustainable agriculture.

Arbuscular Mycorrhizal (AM) fungi are considered important in growth and development of plants. This is brought about by enhanced uptake of phosphorus [3, 4] and also other diffusion-limited elements like Zn, Cu etc. [5] by increasing the nutritional status of the host. AM fungi are also involved in increasing the uptake of water [6, 7] and in protecting the plants from phytopathogenic fungi, bacteria and parasitic nematodes invading the roots [8].

AM fungi enhancing the activity of beneficial soil organisms, like nitrogen fixers and phosphate solubilizers with consequential beneficial effect on plant growth has been reported [9]. *Trichoderma* spp. are known to induce plant growth by producing growth regulating factors [10] and suppressing the activity of pathogenic organisms [11]. They are also known to solubilize unavailable form of P to a form available for plant growth [12]. Recently it was found to enhance the activity of AM fungus and hence was designated as

Corresponding Author: Dr. D.J. Bagyaraj, Department of Agricultural Microbiology, University of Agricultural Sciences, G.K.V.K. Campus, Bangalore 560065, India

Mycorrhiza Helper Organism (MHO) [13]. But the information available on the interaction between AM fungi and *Trichoderma* and their consequential effect on plant growth is meager. Recently, it was reported that dual inoculation with *Glomus mosseae and Trichoderma viride* increased the yield and forskolin content in *Coleus forskohlii* [14]. The present study was undertaken to understand the response of kalmegh to the AM fungus *Glomus mosseae* and the Plant Growth Promoting Rhizomicroorganism (PGPR)/MHO, *Trichoderma harzianum*, at the recommended level of P and 75% of the recommended P, under field condition.

MATERIALS AND METHODS

A microplot investigation was conducted to examine the response of kalmegh *Andrographis paniculata* Nees. to inoculation with the AM fungus *G. mosseae* and the Plant Growth Promoting Rhizomicroorganism (PGPR)/MHO *T. harzianum* at two levels of P fertilizer application. The experimental plot was prepared and brought to fine tilth. The soil was an alfisol of a fine kaolinitic, isohyperthermic typic kanhaplustafs type with a pH of 5.93, organic carbon of 0.56 g per 100 g solution and available phosphorus of 25 kg ha^{-1} (Brays method). The net plot size was 1×1 m with proper irrigation channels. Well decomposed farm yard manure at the rate of 2.5 kg per plot was applied and mixed thoroughly. Similarly the recommended dose of N (75 kg ha^{-1}) and K (41 kg ha^{-1}) were applied in the form of urea and muriate of potash respectively, to all plots. P was added in the form of single super phosphate according to the treatment level, the recommended level being 22 kg ha^{-1}. Half the dose of N along with full dose of P and K were applied at the time of planting, while the remaining half of N was applied four weeks later. The AM fungus *Glomus mosseae* was maintained in pot culture with sterilized sand: soil 1:1 by vol. as the substrate and Rhodes grass as the host. The inoculum containing substrate with extramatrical hyphae, spores and infected root bits of Rhodes grass was used at the proportion of 10 g per planting point, as per the treatment. The inoculum contained 106 infective propagules per gram of substrate as estimated by 4-fold dilution method [15]. *Trichoderma harzianum* culture was grown in potato dextrose broth for 8 days. The culture was then filtered through Whatman no. 1 filter paper and the mycelial mat was macerated using a waring blender for 1 min and suspended in 0.1 M $MgSO_4.7H_2O$. Ten-ml inoculum containing 5×10^4 c.f.u. ml^{-1} was added to the planting point as per the treatment. Seedlings of kalmegh were first raised in nursery by sowing seeds in polythene bags containing a mixture of soil: sand: FYM in ratio of 1:1:1 by vol. Fifty-day-old seedlings were transplanted to the field as per the following treatments:

T1: Uninoculated control with 100% recommended P (22 kg ha^{-1})
T2: Inoculated with *G. mosseae* at 100% recommended P
T3: Inoculated with *T. harzianum* at 100% recommended P
T4: Inoculated with *G. mosseae* + *T. harzianum* at 100% recommended P
T5: Uninoculated control with 75% recommended P (16.5 kg ha^{-1})
T6: Inoculated with *G. mosseae* at 75% recommended P
T7: Inoculated with *T. harzianum* at 75% recommended P
T8: Inoculated with *G. mosseae* + *T. harzianum* at 75% recommended P

Each treatment had 3 replications. The experiment was laid out as a Randomized Complete Block Design (RCBD). The seedlings were planted with a spacing of 30 cm between rows and 15 cm between plants, with a total of 18 plants/plot. Plots were irrigated twice a week for the first 4 weeks and subsequently at weekly intervals to maintain enough moisture and the plants were raised for 90 days after transplanting.

Plant growth parameters like plant height, plant spread, number of branches/plant, leaf area and days for 50% flowering were recorded at 30 days interval commencing from the Day after Transplanting (DAT). Three such observations were made during entire growth period of the crop. But the data on 90 DAT (i.e. at harvest) alone is presented in this paper. Plant spread was measured along north-south and east-west direction, leaf area was measured using a leaf area meter. After harvest, the plants were dried at 60°C to attain a constant weight to get the plant dry matter (herbage yield)/plot. Phosphorus content in shoot and root was estimated colorimetrically by the vanadomolybdate yellow colour method [16]. The andrographolide concentration in the whole plant was estimated by spectrophotometric method [17].

Fresh root samples were stained using 0.05 g per 100 g solution trypan blue [18] and the percent root colonization was estimated by adapting the gridline intersect method [19]. Extramatrical spores in the root zone soil were enumerated using stereozoom microscope after

wet sieving and decanting the soil samples [20]. Data thus generated were subjected to statistical analysis of variance by RCBD and the treatment means were separated by Duncan's Multiple Range Test (DMRT) [21].

RESULTS AND DISCUSSION

Arbuscular mycorrhizal fungi are well known to enhance the nutritional status of several plants and thereby aid in increased growth and yield. The present investigation was carried out in order to evaluate the role of an AM fungus and a plant growth promoting rhizomicro organism (PGPR)/Mycorrhiza Helper Organism (MHO) on the growth and P nutrition of kalmegh and the possibility of reducing application of phosphate fertilizer through microbial inoculation. Inoculation of kalmegh with *G. mosseae* significantly increased plant height compared to uninoculated plants at both the levels of P (Table 1). Similar results were obtained in the medicinal plant palmarosa [22]. Inoculation with *T. harzianum* also increased plant height compared to uninoculated plants at both the levels of P. Inoculation with *G. mosseae* + *T. harzianum* resulted in higher plant height compared to *T. harzianum* alone, but on par with *G. mosseae* alone. Inoculation with *G. mosseae* + *T. harzianum*, at the two levels of P, significantly increased plant height compared to uninoculated plants. The spread of plants was higher when inoculated with both the organisms, followed by *G. mosseae* alone and *T. harzianum* alone at both the levels of P as compared to uninoculated plants (Table 1).

Number of branches per plant was also significantly larger in inoculated treatments as compared to uninoculated treatments, highest being in the treatment *G. mosseae*+ *T. harzianum*, at both the levels of P (Table 1). Similar trend was observed in the number of leaves per plant and leaf area per plant. Inoculated plants took significantly less number of days for 50 percent flowering as compared to the uninoculated treatments at both the levels of P. At 75% P level, the number of days taken for flowering was significantly less in the treatment *G. mosseae* + *T. harzianum* as compared to treatments with *G. mosseae* or *T. harzianum* alone, both being on par with each other.

Plant dry matter in plants inoculated with both the organisms and plants inoculated with *G. mosseae* alone and with *T. harzianum* alone, at both levels of P, had significantly higher biomass as compared to uninoculated plants (Table 1). The plant dry matter increased by 84.8% when inoculated with both organisms at 100% P followed by plants inoculated with *G. mosseae* alone by 48.4% as compared to the uninoculated plants. When inoculated with both the organisms at 75% P the increase in plant dry matter was by 49.8% followed by *G. mosseae* alone by 28.8% as compared to uninoculated control. Increase in plant biomass because of AM fungal inoculation has been reported in other medicinal plants like Palmarosa [22] and *Coleus forskohlii* [14].

The inoculated treatments with *G. mosseae* increased significantly the P content in both shoot and root as compared to uninoculated plants, at both levels of P (Table 2). Mycorrhizal association is known to increase the availability of diffusion limited nutrients, phosphorus being the most important of these nutrients [23, 24]. Various mechanisms have been suggested for increased P uptake by mycorrhizal plants. The external hyphae of AM fungi allow the root system to exploit greater volume of soil phosphorus (i) by extending away from roots and translocating P from some distance to the root zone [25] and (ii) by exploiting smaller soil pores not reached by root hairs and by adding surface area to the adsorptive system [26]. However, maximum shoot and root P content was observed in plants when *T. harzianum* was inoculated along with *G. mosseae*, at both levels of P,

Table 1: Influence of *Glomus mosseae* (Gm) and *Trichoderma harzianum* (Th) on the plant growth parameters of kalmegh at 90 DAT grown at 100% and 75% recommended level of P fertilizer

Treatments	Plant height (cm)	Plant spread (cm^2)	Branches (No./plant)	Leaves (No./plant)	Leaf area (cm^2/plant)	50% flowering (No. of days)	Dry matter (g/plant)
Uninoculated + 100% P	26.2^{cd}	344.3^{d}	20.6^{e}	43.3^{d}	133.8^{e}	126.6^{a}	154.6^{e}
Inoculated with *G. mosseae* (Gm) at 100% P	33.9^{a}	370.0^{b}	38.0^{a}	56.3^{b}	151.2^{b}	117.0^{cd}	229.5^{b}
Inoculated with *T. harzianum* (Th) at 100% P	26.5^{cd}	355.0^{c}	29.4^{d}	46.0^{c}	145.3^{c}	115.6^{d}	180.4^{d}
Inoculated with Gm + Th at 100% P	32.0^{ab}	375.0^{a}	40.7^{a}	61.0^{a}	155.4^{a}	115.6^{d}	285.7^{a}
Uninoculated + 75% P	24.1^{d}	330.6^{f}	16.5^{f}	34.6^{f}	125.2^{g}	123.3^{b}	142.5^{g}
Gm inoculated + 75% P	31.6^{ab}	347.6^{d}	30.7^{cd}	40.0^{e}	130.4^{f}	118.0^{c}	183.5^{d}
Th inoculated + 75% P	29.0^{bc}	335.0^{e}	28.4^{d}	38.0^{e}	128.4^{g}	119.0^{c}	150.1^{f}
Gm-Th inoculated + 75% P	31.9^{ab}	357.3^{c}	33.4^{bc}	44.6^{cd}	138.3^{d}	114.3^{e}	213.4^{c}

Means followed by the same letter within a column do not differ significantly at p#0.05 by Duncan's Multiple Range Test, DAT= Days after transplanting

Table 2: Influence of *Glomus mosseae* (Gm) and *Trichoderma harzianum* (Th) on the P content and andrographolide concentration of kalmegh at 90 DAT, grown at 100% and 75% recommended level of P fertilizer

	P content (mg/plant)		
Treatments	Shoot	Root	Andrographolide concentration (%)
Uninoculated + 100% P	4.4^{e}	2.4^{e}	1.3^{c}
Inoculated with *G. mosseae* (Gm) at 100% P	7.0^{c}	4.1^{c}	1.9^{ab}
Inoculated with *T. harzianum* (Th) at 100% P	5.5^{d}	2.7^{e}	1.4^{b}
Inoculated with Gm + Th at 100% P	21.2^{a}	9.0^{a}	2.1^{a}
Uninoculated + 75% P	4.0^{e}	2.5^{e}	1.1^{c}
Gm inoculated + 75% P	5.5^{d}	3.7^{d}	1.5^{bc}
Th inoculated + 75% P	4.3^{e}	2.9^{e}	1.4^{bc}
Gm-Th inoculated + 75% P	15.3^{b}	8.1^{b}	2.1^{a}

Means followed by the same letter within a column do not differ significantly at P#0.05 by Duncan's Multiple Range Test, DAT = Days after transplanting

Table 3: Influence of *Glomus mosseae* (Gm) and *Trichoderma harzianum* (Th) on mycorrhizal root colonization, spore numbers and population of *T. harzianum* in the root zone soil of kalmegh at 90 DAT grown at 100% and 75% recommended level of P fertilizer

Treatments	Colonization (%)	Spore number/50g of soil	*T. harzianum* (Xx10^{5} c.f.u /g soil)
Uninoculated + 100% P	25.3^{e}	48.3^{c}	ND
Inoculated with *G. mosseae* (Gm) at 100% P	45.3^{c}	105.0^{a}	ND
Inoculated with *T. harzianum* (Th) at 100% P	22.3^{f}	56.0^{c}	1.1
Inoculated with Gm + Th at 100% P	52.0^{a}	123.0^{a}	6.6
Uninoculated + 75% P	22.0^{f}	42.3^{c}	ND
Gm inoculated + 75% P	40.0^{d}	92.3^{b}	ND
Th inoculated + 75% P	25.0^{e}	55.6^{c}	1
Gm-Th inoculated + 75% P	48.0^{b}	112.3^{a}	5.6

ND = Not Determined, Means followed by the same letter within a column do not differ significantly at P#0.05 by Duncan's Multiple Range Test, c.f.u = colony forming units, DAT = Days after Transplanting

which was as compared to plants inoculated with *G. mosseae* alone (Table 2). The least uptake was observed in uninoculated plants. *T. harzianum* is known to solubilize unavailable forms of P and convert them to an available form [12] thus enabling better P uptake by AM fungi [27, 28].

Both the organisms when inoculated singly increased the andrographolide concentration of the plant as compared to uninoculated plants. At the recommended level of P, dual inoculation increased slightly the andrographolide concentration as compared to plants inoculated with *G. mosseae* alone but statistically they were on par with each other. But at 75% recommended P level, the andrographolide concentration in plants inoculated with *G. mosseae* + *T. harzianum* was significantly larger as compared to plants treated singly with *G. mosseae or T. harzianum* (Table 2). Higher curcumin content in *Curcuma longa* because of inoculation with *Azotobacter* and *Azospirillum* [29] and larger forskolin concentration in *Coleus forskohlii* plants inoculated with *Glomus mosseae* + *Trichoderma viride* [14] were reported earlier under field conditions.

All the inoculated treatments except *T. harzianum* at 75% P level, increased significantly the percentage mycorrhizal root colonization as compared to uninoculated plants (Table 3). Among the single inoculated treatments, highest mycorrhizal colonization was observed in plants inoculated with the AM fungus *G. mosseae*, thus supporting the well-documented fact that inoculation with effective AM fungi enhances mycorrhizal root colonization [30]. Highest mycorrhizal root colonization in kalmegh was observed when *G. mosseae* was co-inoculated with *T. harzianum* at both levels of P. The least mycorrhizal root colonization was observed in uninoculated plants. A similar trend was observed in the mycorrhizal spore numbers in the root zone soil (Table 3). This may be because of synergistic interaction between AM fungus and PGPR/MHO supporting earlier reports [13, 14]. The stimulation was attributed to the volatile compounds produced by *Trichoderma* spp. [7]. Enhanced mycorrhizal root colonization and sporulation by *G. mosseae* because of co-inoculation with *T. harzianum* upholds this fungus in the category of MHO as reported earlier [27].

Population of *T. harzianum* was found to increase in plants inoculated with both the organisms. The colony-forming-units of *T. harzianum* were higher when both organisms were inoculated together and least when inoculated alone (Table 3). Increased population of *T. harzianum* was observed when micropropagated ficus and sugarcane were co-inoculated with *G. mosseae* as compared to single inoculation with *T. harzianum* [27].

The results of the present study clearly brought out the beneficial effect of inoculation with *Glomus mosseae* plus *Trichoderma harzianum* on the growth parameters like plant height, number of branches, plant spread, number of days taken for 50% flowering, P nutrition, yield and andrographolide concentration of an important medicinal plant, cultivated commercially. Further, through this *G. mosseae-T. harzianum* co-inoculation, application of phosphatic fertilizer can be reduced by 25 percent.

REFERENCES

1. Weibo, L., 1995. Prospects for study on treatment of AIDS with traditional Chinese medicine. J. Traditional Chinese Med., 15: 3-9.
2. Matsuda, T., M. Kuroyanagi, S. Sugiyama, K. Umehara, A. Ueno and K. Nishi, 1994. Cell differentiation inducing diterpenes from *Andrographis paniculata* Nees. Chem. Pharm. Bull., 42: 1216-1225.
3. Sanders, F.E. and P.B. Tinker, 1971. Mechanism of absorption of phosphate from soil by *Endogone* mycorrhizas. Nature, 233: 278-379.
4. Rhodes, L.H. and J.W. Gerdemann, 1978. Influence of phosphorus nutrition on sulphur uptake by vesicular arbuscular mycorrhiza by onion. Soil Biol. Biochem., 10: 361-368.
5. La-Rue, J.W., W.A. McClellen and W.L. Peacock, 1975. Mycorrhizal fungi and peach nursery nutrition. California Agric., 29: 6-7.
6. Allen, M.F., 1982. Spore germination and axenic culture of endomycorrhizae. In: Methods and Principles of Mycorrhizal Research. Ed., Schenck, NC. st. Paul: American Phytopathological Society Pub., pp: 85-90.
7. Graham, J.H. and J.P. Sylvertsen, 1984. Influence of vesicular arbuscular mycorrhizae on the hydraulic conductivity of roots of two citrus rootstocks. New Phytol., 7: 277-284.
8. Bagyaraj, D.J., 1984. Biological interactions with VAM fungi. In: *Mycorrhiza*, Powell, C.L. and D.J. Bagyaraj (Eds.). Boca Raton: CRC Press. ISBN: 0-8493-5694-6, pp: 131-153.
9. Lindermann, R.G, 1992. Vesicular-arbuscular mycorrhizae and soil microbial interactions. In: Mycorrhizae in Sustainable Agriculture. Bethlenfalvay, G.J. and R.G. Linderman, (Eds.). Madison, USA: ASA Publication. ISBN: 0-89118-112-1, pp: 45-68.
10. Baker, R., T. Paulitz, M.H. Windham and Y. Elad, 1986. Enhancement of growth of ornamentals by a biological control agent. Colorado Growers Association Research Bulletin, 431: 1.
11. Michrina, J., A. Michalikova, T. Konacik and R. Kulichora, 1995. Antibiosis, a possible mechanism of antagonistic action of *Trichoderma harzianum* against *Fusarium culmorum*. Ocharna Rostlin, 31: 177-184.
12. Anusuya, D. and R. Jeyarajan, 1998. Phosphorus solubilization by *Trichoderma viride*. Current Sci., 74: 264-265.
13. Jayanthi Srinath, D.J. Bagyaraj and B.N. Satyanarayana, 2003. Enhanced growth and nutrition of micropropagated *Ficus benjamina* to *Glomus mosseae* co-inoculated with *Trichoderma harzianum* and *Bacillus coagulans*. World J. Microbiol. Biotechnol., 19: 69-72.
14. Boby, V.U. and D.J. Bagyaraj, 2003. Biological control of root rot of *Coleus forskohlii* Briq. using microbial inoculants. World J. Microbiol. Biotechnol., 19: 175-180.
15. Sieverding, E., 1991. Vesicular-arbuscular Mycorrhizal Management in Tropical Agro-systems. Federal Republic of Germany, Friedland: Technical Co-operation, Bremer.
16. Jackson, M.L, 1973. Soil Chemical Analysis. New Delhi: Prentice Hall (India) Pvt. Ltd., pp: 239-241.
17. Gaind, K.N., R.N. Dar and R.N. Kaul, 1963. Spectrophotometric method of assay of andrographolide in Kalmegh. Ind. J. Pharm., 25: 225-226.
18. Philips, J.H. and D.S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycolog. Soc., 55: 158-161.

19. Giovannetti, M. and B. Mosse, 1980. An evaluation of technologies for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol., 84: 489-500.
20. Gerdemann, J.W. and T.H. Nicolson, 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc., 46: 235-244.
21. Little, T.M. and F.J. Hills, 1978. Agricultural Experimentation: Design and Analysis. USA. John Wiley and Sons. Inc. ISBN: 0-4710-2352-3.
22. Gupta, M.L. and K.K. Janardhanan, 1991. Mycorrhizal association of *Glomus aggregatum* with palmarosa enhances growth and biomass. Plant and Soil, 131: 261-264.
23. Black, R.L.B. and P.B. Tinker, 1997. Interaction between effects of vesicular arbuscular mycorrhizal fungi and fertilizer phosphorus on yields of potatoes in the field. Nature, 267: 510-511.
24. Sreeramulu, K.R. and D.J. Bagyaraj, 1999. Screening of arbuscular mycorrhizal fungi in cardomom cv. Mysore. J. Plant. Crops, 27: 207-211.
25. Rhodes, L.H. and J.W. Gerdemann, 1975. Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol., 75: 555-561.
26. Okeffe, D.M. and S.E. Sylvia, 1990. Mechanisms of VA mycorrhiza plant growth response. In: Handbook of Applied Mycology Vol. 1. Arora, D.K., B.R. Rai, K.G. Mukerji, G.R. Knudsen (Eds.), New York, Dekker, pp: 35-54.
27. Jayanthi Srinath and D.J. Bagyaraj, 1998. Response of micropropagated sugarcane to inoculation with *Glomus mosseae, Trichoderma harzianum and Bacillus coagulans*. J. Soil Biol. Ecol., 18: 23-29.
28. Sumana, D.A. and D.J. Bagyaraj, 2003. Influence of VAM fungi on growth response of Neem (*Azadirachta indica* A. Juss)-Secondary screening. J. Trop. Forest Sci., 15: 531-538.
29. Sena, M.K. and P.K. Das, 1998. Influence of microbial inoculants on quality of turmeric. India cocoa, Arecanut and Spices J., 21: 31-33.
30. Rajan, S.K., B.J.D. Reddy and D.J. Bagyaraj, 2000. Screening of arbuscular fungi for their symbiotic efficiency with *Tectona grandis*. Forest Ecol. Manage., 126: 91-95.
31. Calvet, C., J.M. Barea and J. Pera, 1992: *In vitro* interactions between the vesicular arbuscular fungus *Glomus mosseae* and some saprophytic fungi isolated from organic substrates. Soil Biol. Biochem., 24: 775-780.

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 39-47, 2007
ISSN 1818-6769

Hydrology and Water Quality Assessment of the Tasik Chini's Feeder Rivers, Pahang, Malaysia

G. Muhammad-Barzani, B.S. Ismail, Sahibin Abd. Rahim, Sujaul-Islam Mir and C.C. Tan

Faculty Science and Technology, School of Environmental and Natural Resource Sciences, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

Abstract: The purpose of the study was to assess the hydrological properties and water quality of the seven feeder rivers of Tasik Chini, Pahang, Malaysia. The study was carried out in October and December 2004 and in February, March and April 2005. A total of nine sampling stations were selected for this study: Datang River, Cenahan River, downstream Gumum River, central Gumum River, Kura-kura River, Melai River, downstream mouth of Merupuk River, upstream mouth of Merupuk River and Jemberau River. Eleven water quality parameters were analyzed based on *in-situ* and *ex-situ* analysis during two season periods. Laboratory analysis was carried out according to the HACH and APHA methods. *In situ* water quality findings were as follows: pH (3.2-6.32), dissolved oxygen (0.27-6.4 mg l^{-1}), conductivity (14.33-85.7 µS cm^{-1}) and temperature (24.07-32.1°C). For *ex-situ* water quality parameters, results of TDS ranged from 22.67 to 184 mg l^{-1}, TSS (1.17-79.11 mg l^{-1}) and turbidity (4.67-28.67 NTU) and nutrients (ammonical nitrogen: 0.007 to 0.57 mg l^{-1}; nitrate: 0.7 to 2.9 mg l^{-1}; phosphate: 0.0 to 0.50 mg l^{-1} and sulphate: 0.0 to 2.0 mg l^{-1}). Stream flows were determined during sampling to range from 0.0042 to 0.9083 m^3 sec^{-1} or, on average, 0.1674 m^3 sec^{-1}. The annual rainfall for the lake ranges from 1487.7 to 3071.4 mm. Recent activities such as illegal logging, agricultural activities and other unsustainable developments have taken place in the areas surrounding the lake. The impact of these activities may have caused environmental degradation to Tasik Chini and its adjacent areas by changing the water system's hydrological characteristics, with prospects of possible long term deterioration.

Key words: Water quality parameters % feeder river % hydrological characteristics % Tasik Chini

INTRODUCTION

Surface water resources have played an important function throughout the history in the development of human civilization. About one third of the drinking water requirement of the world is obtained from surface sources like rivers, canals and lakes [1]. Unfortunately, these sources seem to be used as convenient places for the discharge of domestic as well as agricultural and domestic wastes. Dams, according to UNEP [2], are a visible tool for managing freshwater resources, contributing to socio-economic development and protecting drinking water supply. However, dams may negatively affect changes in downstream water flows, degradation of water quality, increased in-lake sedimentation, lake and river bank scouring, blocked movement of migratory species and loss of aquatic biodiversity. Tasik Chini and its environment has undergone devastating changes since 1984 or earlier brought about by development in surrounding areas through mining, oil palm plantation and urbanization. Tasik Chini was once well-known as rich in biological sources. A study carried out by the MNS [3] found 288 species of plants, 21 species of aquatic plants and 92 species of birds and 144 species of freshwater fishes at the Tasik Chini environment.

The condition of Tasik Chini worsened when a small dam was built in 1995 to retain water in Tasik Chini for tourism purposes [4]. The dam made water movement more sluggish. Fishing activities were affected and water current was unstable. However, lake water has been declared safe for recreation purposes [5]. In this study, water quality was examined, along with various physico-chemical parameters, to determine the factors contributing to the pollution load of the lotic water bodies in and around the Tasik Chini. A better understanding of the hydrology of the Tasik Chini will promote the development and management of the lake environment in a more sustainable way.

Corresponding Author: Dr. B.S. Ismail, Faculty Science and Technology, School of Environmental and Natural Resource Sciences, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

MATERIALS AND METHODS

Study area: Tasik Chini is located in the southeast region of Pahang, Malaysia. The lake system lies at 3° 15´ 40"N and 102° 45' 40"E and comprises 12 open water bodies. The area has a humid tropical climate with two monsoon periods, characterized by a bimodal pattern: southwest and northeast monsoons bring annual rainfall from 1488 to 3071 mm. However, the open water area has expanded greatly since 1995, due to increased retention of water after the construction of a barrage at Chini River. Tasik Chini is surrounded by variously vegetated low hills and undulating lands which constitute the watershed of the region. Three hill areas surround the lake area; (1) Ketaya Hill (209 m) located at the southeast; (2) Tebakang Hill (210 m) at the northern and (3) Chini Hill (641m) at the southeast. The lake drains northeasterly into Pahang River via the Chini River. The lake is drained by the Chini River, which meanders for 4.8 km before it reaches the Pahang River.

Sampling and preservation: Global Positioning System (GPS) was used to determine the actual coordinates of the sampling stations and to re-confirm the location of stations during the subsequent sampling periods. Nine sampling stations, selected during the first trip to Tasik Chini, were established as the main feeder rivers of Tasik Chini: Datang River (Station 1), Cenahan River (Station 2), downstream Gumum River (Station 3), central Gumum River (Station 4), Kura-kura River (Station 5), Melai River (Station 6), downstream at the mouth of the Merupuk River (Station 7), upstream of the mouth of the Merupuk River (Station 8) and Jemberau River (Station 9). Surface water was collected from each station for measurement of concentration levels using standard laboratory methods [6]. Surface water samples were collected about 10 cm below the water surface using a HDPE bottle (500 ml). The samples were stored in an icebox and transported back to the laboratory for analysis on the same day. Total rainfall during the study period was obtained from the nearby weather station at Chini 2, while rainfall data before year 2004 were obtained from Meteorological Department at Petaling Jaya, Selangor, Malaysia.

Analytical methods: The temperature, electrical conductivity, dissolved oxygen and pH of the water samples were measured in the field by *in-situ* measurement. Bottle samples were measured by laboratory analysis for turbidity, TSS, TDS, NH_3-N, NO_3G, $PO_4^{3!}$ and $SO_4^{2!}$. Total Suspended Solids (TSS) was measured using filtration methods with 45 µm membrane filter and vacuum pump (gravimetric methods). Total Dissolved Solids (TDS) was measured using sample water after filtration; turbidity was measured by spectrophotometer. Four chemical water quality parameters (NH_3-N, $NO_3^!$, $PO_4^{3!}$ and SO_4^{26}) were determined by the salicylate method (HACH kit DR 2010). Current flow and river width were measured by flow meter (model FP101) and Rangefinder (model Bushnell 20-0001) was used to measure the distance.

RESULTS AND DISCUSSION

Hydrology: Hydrological analysis was carried out to determine the water level of the water body and of its drainage systems [7, 8]. Between 1994 and 1997, annual total rainfall for the Chini area ranged from 1487.7 (1997) mm to 3071.4 mm (1994) (Fig. 1). The average rainfall was 2235 mm/year or 186 mm/month. The total annual rain days in the study area ranged from 154 to 197, for an average of 178 days/year or 15 days/month. The highest total rain days were observed in 1993 and 1994 (197 and 190 days, respectively), while 1997 saw the fewest total rain days-154 (Fig. 2). The total rain days during 2004 were 159 (Fig. 3). The highest number of rain days per

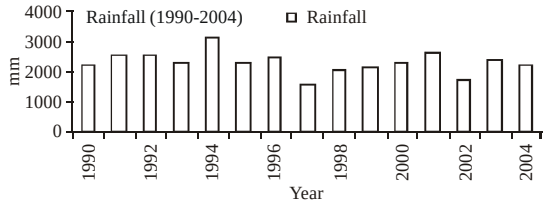

Fig. 1: Distribution of rainfall from 1990 to 2004

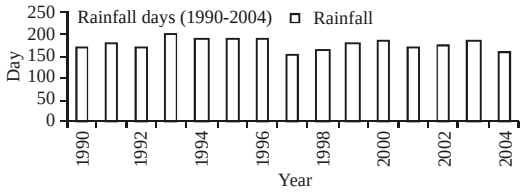

Fig. 2: Distribution of rain days from 1990 to 2004

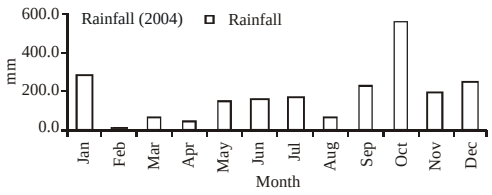

Fig. 3: Distribution of rainfall from January to December 2004

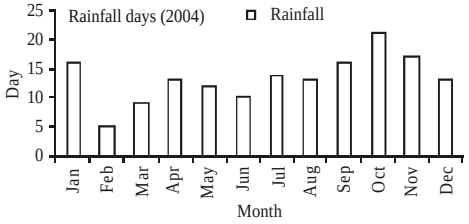

Fig. 4: Distribution of rain days from January to December 2004

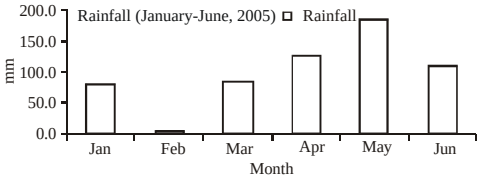

Fig. 5: Distribution of rainfall from January to June 2005

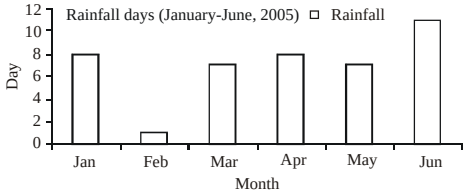

Fig. 6: Distribution of rain days from January to December 2005

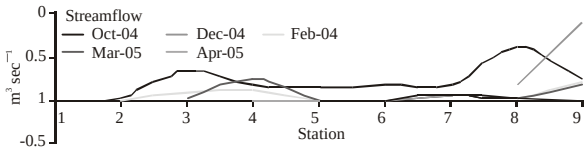

Fig. 7: Stream flow distribution in 9 sampling stations

month (21 days) was recorded during the wet season in 2004, during October to December, while February recorded the lowest number of rain days (5 days) in 2004. The highest rainfall (553.5 mm) was recorded in October 2004; the lowest (16.2 mm) was recorded in February 2004 (Fig. 4). The total annual rainfall was 2192 mm in 2004. During the first half of 2005, total monthly rainfall ranged from 5.3 mm (February) to 182.9 mm (May), for an average of 98 mm/month (Fig. 5). Total rain days for the same period ranged from 1 day (Feb) to 11 days (June) or an average of 7 days/month (Fig. 6).

Stream flow: Stream flow from each feeder river of Tasik Chini is relatively low, ranging from 0.0042 to 0.9083 m^3 sec^{-1} or, on average, 0.1674 m^3 sec^{-1} (Fig. 7). Daily discharge ranged from 362.88 to 78,477.12 m^3 or, on average, 14,463.36 m^3/daily. Stream flow of the Kuala Merupuk River was the highest (0.9083 m^3 sec^{-1}) and Melai River was the lowest (0.0042 m^3 sec^{-1}). Datang River is considered a dead river because no water is flowing. Similar inferences have been made on earlier observations in different feeder rivers of Tasik Chini [9, 10]. The ranges of discharge values from feeder rivers based on each sampling are as follow: 0.033 to 0.6166 m^3 sec^{-1} in October 2004, 0.172 to 0.9083 $m^3 sec^{-1}$ in December 2004, 0.0118 to 0.207 m^3 sec^{-1} in February 2005, 0.0042 to 0.2448 m^3 sec^{-1} in March 2005 and 0.0029 to 0.0718 m^3 sec^{-1} in April 2005.

Average monthly measurement of stream flow of all nine feeder rivers of Tasik Chini in October and December 2004 and February, March and April 2005 were, respectively 0.2162, 0.5308, 0.0624, 0.0655 and 0.0157 m^3 sec^{-1}. Stream flows of these rivers mainly depend on rainfall. The highest stream flows were observed during the wet season, especially in October 2004 (0.6166 m^3 sec^{-1}) and December 2004 (0.9083 m^3 sec^{-1}), while in the dry season (February to April 2005) we recorded the lowest stream flows (0.0118, 0.0042 and 0.0029 m^3 sec^{-1}). Data could not be obtained from the Gumum, Kura-kura, Melai and Kuala Merupuk Rivers in December 2004 due to flooding.

Water quality: Figure 8 shows the water quality parameters *viz*. temperature, pH, conductivity, DO, TDS, TSS, turbidity, ammonical nitrogen, nitrate, phosphate and sulphate.

Temperature: Range of temperature value based on each sampling as described as follow: 24.07-25.47°C in October 2004, 24.87-27.97°C in December 2004, 24.87-28.4°C in February, 24.3-29°C in March 2005 and 24.57-32.1°C in April 2005 (Fig. 8). For all sampling stations, temperature of the water ranged from 24.07 to 32.1°C. Station 7 (downstream of the Kuala Merupuk River) recorded the lowest value (24.07°C) in the wet season and station 9 (Jemberau River) recorded the highest value (32.1°C) in the dry season. The range of temperature at these sampling sites during the different seasons seemed normal for the climate. The temperature values did not show any spatial change but indicated temporal variation.

pH: The ranges of pH value in the different sampling times were recorded: 4.96 to 6.32 in October 2004, 5.15 to 5.94 in December 2004, 4.17 to 5.39 in February 2005, 3.2 to 5.46 in March 2005 and 4.24 to 5.82 in April 2005 (Fig. 8). pH values ranged from 3.2 at Station 4 (central Gumum

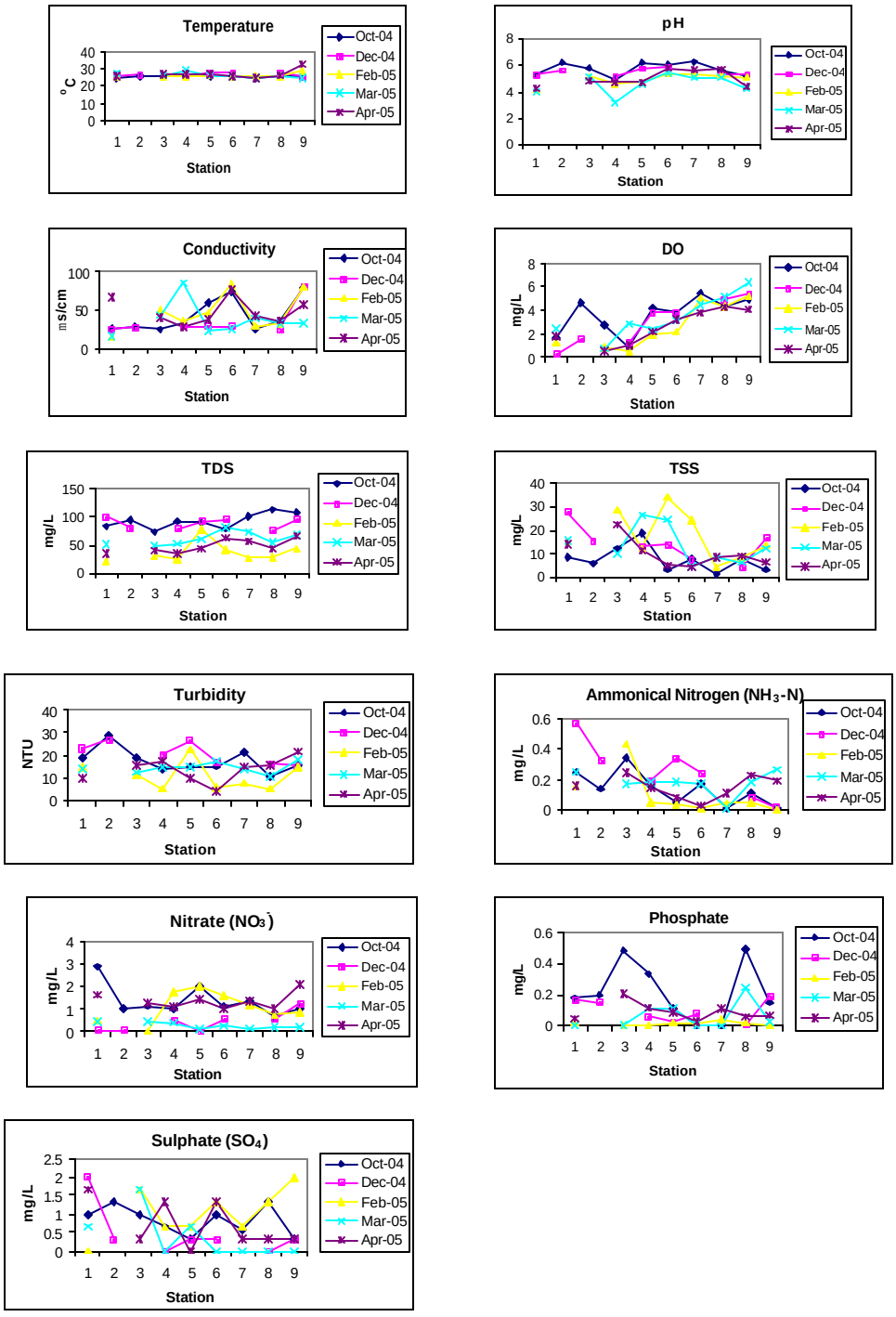

Fig. 8: Distribution of 11 water quality parameters; i.e. Temperature; pH; Conductivity; DO; TDS; TSS; Turbidity; Ammonical nitrogen; Nitrate; Phosphate and Sulphate

River) to 6.32 at Station 7 (downstream Kuala Merupuk River). Most stations showed slightly acidic pH, indicating that the water is in Class III according to Interim National Water Quality Standards (INQWS) [11]. It is clear that, the pH values increased from the dry season to the wet season. Our findings are similar to pH values found by Sim [12]. The pH value is controlled by the dissolved carbon dioxide (CO_2), which forms carbonic acid in water [13]. The INWQS threshold range of pH for Malaysian rivers is 5.00 to 9.00 [11].

Conductivity: Conductivity (EC) values ranged from 14.33 µS cm^{-1} to 85.7 µS cm^{-1} at different locations and times, as indicated by the *in situ* readings obtained during sampling (Fig. 8). The average value was 40.96 µS cm^{-1}. The ranges of conductivity were recorded: 24.83 to 80.33 µS cm^{-1} in October 2004, 25.3 to 81.4 µS cm^{-1} in December 2004, 14.33 to 84.7 µS cm^{-1} in February 2005, 16.5 to 85.7 µS cm^{-1} in March 2005 and 27.93 to 76.43 µS cm^{-1} in April 2005. The highest and lowest values were recorded respectively at Station 4 (14.33 µS cm^{-1}) in March 2005 and station 1 (85.7 µS cm^{-1}) in February 2005. Conductivity values were higher compared to the ranges 13.2 to 25.13 µS sec^{-1} found by Sim [12].

Dissolved oxygen: The dissolved oxygen (DO) concentration ranged from 0.56 to 6.4 mg l^{-1} (Fig. 8). The DO value did not show any difference between wet and dry seasons. The range of DO values measured monthly were recorded: 0.88 to 5.48 mg l^{-1} in October 2004, 1.23 to 5.37 mg l^{-1} in December 2004, 0.59 to 5.16 mg l^{-1} in February 2005, 0.72 to 6.4 mg l^{-1} in March 2005 and 0.56 to 4.21 mg l^{-1} in April 2005. The highest value (6.4 mg l^{-1}) was recorded at Jemberau River in March 2005 and the lowest value (0.56 mg l^{-1}) occurred at downstream Gumum River during the dry season (April 2005). The DO value was very low (0.56 to 0.88 mg l^{-1}) at downstream Gumum River during the dry season (February to April 2005). Our findings on DO are similar to those obtained in earlier observations in different feeder rivers of Tasik Chini [14]. The DO values are higher (4.03 to 6.4 mg l^{-1}) at Jemberau River during both wet and dry seasons. The water flows of the Jemberau River during both wet and dry seasons were higher, providing more oxygen to dissolve into the water. The threshold range for Malaysian rivers is 3.00 to 5.00 mg l^{-1} [11].

Total dissolved solids: The range of total dissolved solids (TDS) values recorded in each monthly sampling were: 73.33 to 112.76 mg l^{-1} in October 2004, 75.33 to 100.67 mg l^{-1} in December 2004, 22.67 to 78.33 mg l^{-1} in February 2005, 50 to 8.67 mg l^{-1} in March 2005 and 35.33 to 66.67 mg l^{-1} in April 2005 (Fig. 8). TDS of water samples collected during different seasons varied from 22.67 to 112.67 mg l^{-1}, well within the permissible limits of the World Health Organization [15]. The highest concentration (112.67 mg l^{-1}) was measured at the upstream Kuala Merupuk River (Station 8) during the wet season and the lowest value (22.67 mg l^{-1}) was recorded at the Datang River during the dry season (February 2005). According to INQWS, all the feeder rivers are in Class I (TDS<500 mg l^{-1}). In general, TDS increased from dry to wet seasons. In the dry season (February to April 2005) TDS ranged from 22.67 to 80.67 mg l^{-1} and in the wet season (November to December 2004) TDS ranged from 73.33 to 112.67 mg l^{-1}. The TDS values were always higher at the Jemberau River (Station 9), across both wet and dry seasons TDS ranged 45.33 to 108 mg l^{-1}.

Total suspended solids: The Total Suspended Solids (TSS) of water samples collected from 7 feeder rivers during the different seasons varied from 1.17 to 34.0 mg l^{-1} (Fig. 8). The mean concentration of TSS was 12.27 mg l^{-1}; the highest (34.0 mg l^{-1}) was recorded at the Kura-kura River (Station 5) during the dry season and the lowest (1.17 mg l^{-1}) at the Kuala Merupuk River (Station 7) during the wet season. The ranges of monthly measurement of TSS in the different seasons were recorded: 1.17 to 19 mg l^{-1} in October 2004, 4.25 to 27.83 mg l^{-1} in December 2004, 4.0 to 34.0 mg l^{-1} in February 2005, 4.5 to 26.67 mg l^{-1} in March 2005 and 4.17 to 22.5 mg l^{-1} on April 2005. The TSS values were comparatively higher at the Gumum River during both wet and dry seasons. There was sudden rise in the TSS values of the Gumum River (Stations 3 and 4) in February 2005 and March 2005, respectively. The TSS value also rose at the Kura-kura River (Station 5) in February 2005 and March 2005. Overall, TSS concentrations recorded in this study show a low value. The INWQS recommends maximum threshold levels of TSS for Malaysian rivers from 25 to 50 mg l^{-1}. The INQWS threshold level of TSS for supporting aquatic life in fresh water ecosystems is 150 mg l^{-1} [11].

Turbidity: The turbidity of water samples varied from 4.67 to 28.67 NTU (Fig. 8). The mean concentration was 16.41 NTU; the highest was 28.67 NTU at the Cenahan River (Station 2) during the wet season and the lowest was 4.67 NTU at the Melai River (Station 6) during the dry

season. The ranges of turbidity in each sampling period were: 11.0 to 28.67 NTU in October 2004, 16.0 to 27.33 NTU in December 2004, 5.33 to 22.67 NTU in February 2005, 10.67 to 18.33 NTU in March 2005 and 4.67 to 21.67 NTU in April 2005. Overall, during the wet season turbidity was higher than in the dry season. The highest turbidity value was measured at the Kura-kura River during both wet and dry seasons. According to international standards, water is acceptable for domestic use when its turbidity lies within 5-25 NTU [16]. INWQS does not propose any threshold level for turbidity of fresh waters for the support of aquatic life. The Ministry of Health has set a threshold level of raw water turbidity at 1000.00 NTU.

Ammonical nitrogen (NH_3-N): The ranges of ammonical nitrogen at each sampling are as follows: 0.007 to 0.34 mg lG^1 in October 2004, 0.013 to 0.57 mg lG^1 in December 2004, 0.003 to 0.43 mg lG^1 in February 2005, 0.014 to 0.26 mg lG^1 in March 2005 and 0.03 to 0.24 mg lG^1 in April 2005 (Fig. 8). The value of ammonical nitrogen of all water samples collected ranged from 0.003 to 0.57 mg lG^1. The highest concentration (0.57 mg lG^1) was observed at the Datang River (Station 1) during the wet season. The lowest (0.003 mg lG^1) was recorded at the Jemberau River (Station 9) in February 2005 during the dry season. The average concentration of ammonical nitrogen was 0.17 mg lG^1. All the samples collected during the dry season were well below the maximum permissible limit set by the World Health Organization [15]. Even samples collected during the wet season did not exceed the WHO limit. The INWQS recommends maximum threshold levels of ammonical nitrogen for Malaysian rivers at 0.90 mg lG^1 to support aquatic life.

Nitrate ($NO_3^!$): The range of nitrate values recorded were 0.7 to 2.9 mg lG^1 in October 2004, 0.0 to 1.27 mg lG^1 in December 2004, 0.0 to 2.03 mg lG^1 in February 2005, 0.09 to 0.44 mg lG^1 in March 2005 and 1.03 to 2.1 mg lG^1 in April 2005 (Fig. 8). Nitrate concentrations varied from 0.0 to 2.9 mg lG^1. The $NO_3^!$ ion is usually derived from anthropogenic sources like agricultural fields, domestic sewage and other waste effluents containing nitrogenous compounds [1]. In February 2005, the nitrate concentrations recorded were comparatively low at 0.0 to 0.44 mg lG^1. During the wet season nitrate concentrations were higher, ranging from 0.7 to 2.9 mg lG^1. The nitrate level was recorded zero dowmstream at Gumum River (Station 3) during the dry season. According to the INQWS classification, all the feeder rivers are in Class I, which is considered as not contaminated.

Phosphate: The phosphate levels of water samples measured across the seasons varied from 0.0 to 0.50 mg lG^1 (Fig. 8). The mean phosphate concentration was 0.11 mg lG^1; the highest concentration was 0.50 mg lG^1 recorded at upstream of Kuala Merupuk River (Station 8) during the wet season and the lowest 0.0 mg lG^1 at downstream of Kuala Merupuk River (Station 6) during the dry season. The ranges of phosphate levels across sampling times were recorded as 0.01 to 0.5 mg lG^1 in October 2004, 0.01 to 0.19 mg lG^1 in December 2004, 0.01 to 0.04 mg lG^1 in February 2005, 0.0 to 0.25 mg lG^1 in March 2005 and 0.03 to 0.21 mg lG^1 on April 2005.

Overall, in the wet season phosphate levels were higher than in the dry season. Comparatively higher phosphate concentrations were measured at Gumum River during both seasons, presumably because of human activities at the nearby village of Kampung Gumum. Concentrations of nutrients and pesticides in major rivers reflect the proportion of urban and agricultural land in the drainage basin [17]. Similarly, in the surrounding areas of Gumun River, there are plenty of oil palm plantations with heavy use of fertilizers. The fertilizer may wash to Gumun River and may cause high nutrient contents in the water.

Sulphate: The sulphate content of water samples ranged from 0.0 to 2.0 mg lG^1 (Fig. 8). The highest value (2.0 mg lG^1) was recorded at Datang River (Station 1) during the wet season and the lowest value (0.0 mg lG^1) was recorded during the dry season. The ranges of sulphate levels were: 0.33 to 1.33 mg lG^1 in October 2004, 0.0 to 2.0 mg lG^1 in December 2004, 0.0 to 2.0 mg lG^1 in February 2005, 0.0 to 1.67 mg lG^1 in March 2005 and 0.0 to 1.67 mg lG^1 in April 2005. Across seasons the sulphate levels were higher at Gumun River, again perhaps due to the nearby village of Kampung Gumum and the activities of the local residents. According to Hem [13]. The major sources of sulphate in streams are rock weathering, volcanoes and human activities such as mining, waste discharge and fossil fuel combustion processes. All the samples collected across seasons were well below the maximum permissible limit set by the World Health Organization [15].

Analytical statistics: There are no significant correlations between stream flow and TSS, TDS, turbidity and levels of nitrates and ammonical nitrogen (NH_3-N) during the wet and dry seasons. Most correlations show a very weak R^2 of 0.16 and below (Fig. 9). However, TSS and TDS were correlated as positive and negative slope with stream flow during the wet and the dry season,

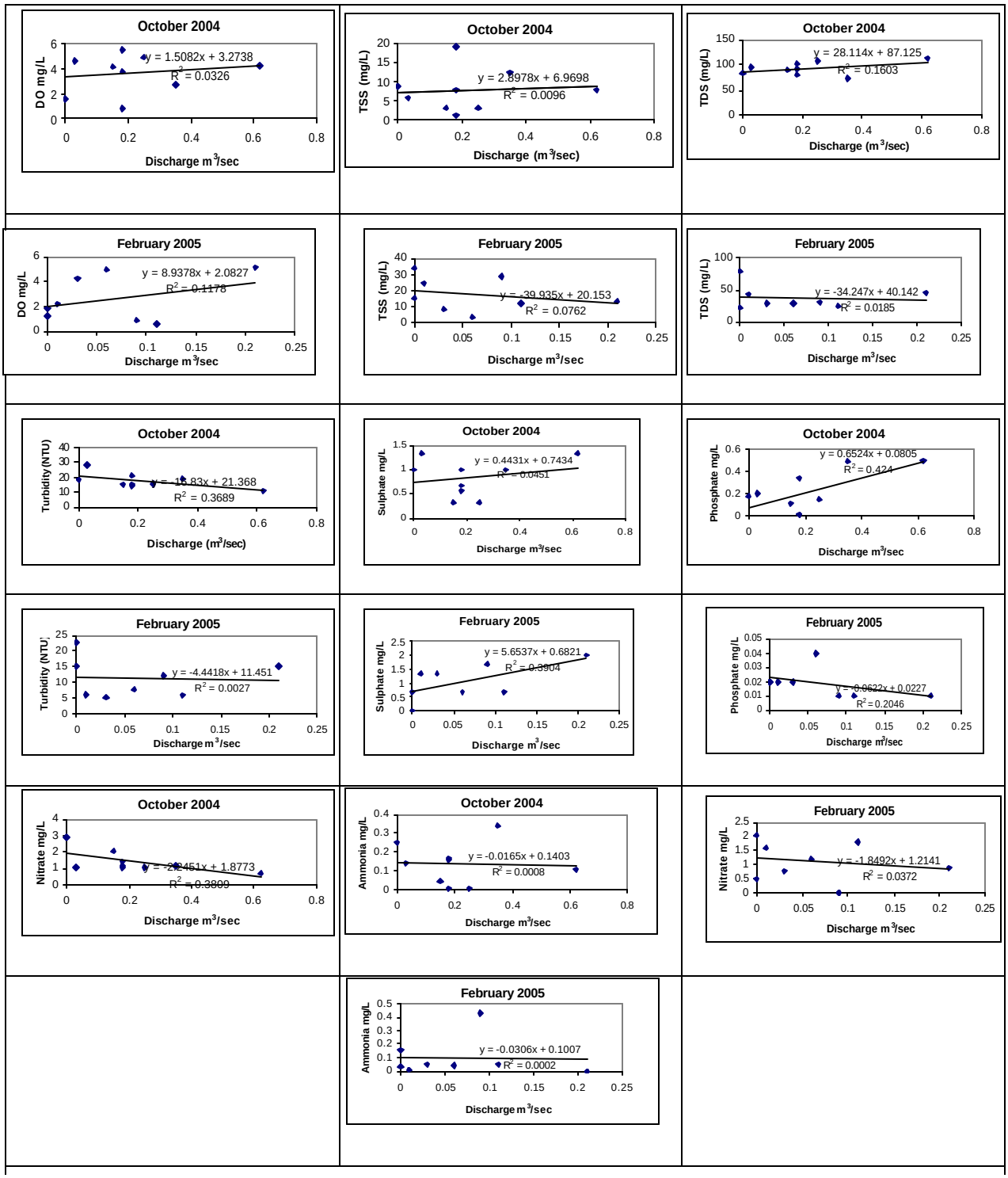

Fig. 9: Relationship between discharge and DO, TDS, TSS, turbidity, Sulphate, Phosphate, Nitrate, and NH_3-N during the wet and dried seasons sampling

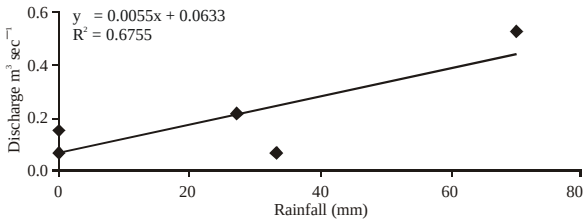

Fig. 10: Relationship between rainfall and discharge

respectively. Levels of DO and sulphate were correlated as positive slope and turbidity and nitrate as negative slope with stream flow during both seasons. Levels of phosphate and sulphate were positively correlated ($R^2 = 0.424$; $R^2 = 0.3904$) with stream flow during wet and dry seasons, respectively. This correlation indicates that pollutant loads probably came from dilution and not from erosion. Raining during the wet season had diluted the soil into the river, increasing the concentration of TSS and turbidity.

Stream flows were recorded during the wet and dry seasons and rainfall data of the earlier five days of sampling days were collected. The measurement of discharge and rainfall showed a statistically significant correlation ($R^2 = 0.6755$) between rainfall and discharge (Fig. 10).

CONCLUSIONS

A detailed physico-chemical study of the lotic water of the feeder river of Tasik Chini during the wet and dry seasons revealed that the seven feeder rivers showed different seasonal fluctuations in various physico-chemical parameters. The results of water quality trends clearly showed that most water quality parameters were quite high in the wet season compared to in the dry season. Water quality analysis shows that pH, NH_3-N, $NO_3^{!}$, phosphate, sulphate, TDS, TSS and turbidity were lower in the dry season, but DO was higher. That is, in the wet season all the parameters were higher except DO. From the above investigation it is clear that Gumum River (Station 3) and Datang River (Station 1) were comparatively more polluted than the other sampling sites, presumably due to human activities in nearby Kampung Gumum. Cenahan River (Station 2), Kura-kura River (Station 5), Melai River (Station 6) and Jemberau River (Station 9) were less polluted. The least polluted river was Kuala Merupuk River (Station 7). The main sources of pollutants were likely to be residential areas, illegal logging, development and agricultural activities, generating both organic and inorganic wastes which ultimately contaminate the water bodies. According to the INWQS classification, all the feeder rivers are in Class II, which is considered as slightly contaminated. Stream flow discharge from each feeder river to Tasik Chini is directly related to rainfall. In the dry season rainfall is low, so discharge from feeder rivers is lower than in the wet season. The feeder rivers' water quality status in the catchment is mainly influenced by the stability of the catchment area. A basin protection strategy comprising development of the monitoring system, assessment of pollution, pollution control and basin conservation should be implemented in order to minimize the impact of changes to the lake areas. If proper attention is not paid to sustainable management of water resources, supervision of logging and raising awareness of local people, then the situation may deteriorate and threaten the environment of Tasik Chini.

ACKNOWLEDGMENT

This study was conducted and supported by the Malaysian government through IRPA grant No. 02-02-09-0117-EA294.

REFERENCES

1. Das, J. and B.C. Acharya, 2003. Hydrology and assessment of lotic water quality in Cuttack City, Ind. J. Water, Air and Soil Pollut., 150: 163-175.
2. United Nations Environment Programme (UNEP), 2003. Freshwater: Meeting our goals, sustaining our future. UNEP Division of Early Warning and Assessment, pp: 35-54.
3. Malaysian Nature Society, 1999. A survey of vegetation and avifauna of Chini. Malaysian Nature Society: Kuala Lumpur.
4. Mushrifah, I. and K. Ahmad-Abas, 2004. Trends of physico-chemical water quality in Tasik Chini. In: The Heritage of Tasik Chini. Mushrifah, I., H. Khatijah and A.M. Lattif, (Eds.). Universiti Kebangsaan Malaysia: Bangi.
5. Ahmad, A., I. Mushrifah, O. Shuhaimi and B. Kamsia, 2004. Study on heavy metals in water and aquatic plants of Tasik Chini. In: The Heritage of Tasik Chini, Mushrifah, I., H. Khatijah and A.M. Lattif, (Eds.). Universiti Kebangsaan Malaysia: Bangi.
6. American Public Health Association (APHA), 1998. Standard methods of water and waste water analysis, (20th Edn.). Washington, DC.

7. Ceballas, A. and S. Schnabel, 1998. Hydrological behavior of a small catchment in the Dehesa land use system. J. Hydrol., 210: 146-160.
8. Gray, D.M., 1970. Handbook on the Principles of Hydrology. National Research Council of Canada: Toronto.
9. Muhammad-Barzani, G., O. Mohd-Shuhaimi and C.C. Tan, 2005. Total flows contribution of the Tasik Chini feeder rivers and its significant water level, Pahang, Malaysia. Proceedings of the 6th ITB-UKM Joint Seminar on Chemistry. Bandung, Indonesia, pp: 543-547.
10. Tan, C.C., G. Muhammad-Barzani and M. Sujaul-Islam, 2005. Hydrology and water quality of feeder rivers, Tasik Chini, Pahang. Proceedings of the National Seminar on Environmental Management. Universiti Kebangsaan Malaysia: Bangi, pp: 122-128.
11. Department of Environment (DOE) Malaysia, 1994. Classification of Malaysian rivers: Methodology and classification of ten rivers. Ministry of Science, Technology and Environment, 2: 4.101-4.104.
12. Sim, C.J., 2004. Comparative study of water quality and heavy metals in selected rivers of Tasik Chini, Pahang. M.Sc. Thesis. Universiti Kebangsaan Malaysia: Bangi.
13. Hem, J.D., 1985. Study and Interpretation of the Chemical Characteristics of Natural Water. (3rd Edn.). Washington: United States Geological Survey.
14. Norlida, M. H., G. Muhammad-Barzani and I. Mushrifah, 2005. Contribution of feeder rivers towards ecohydrology of Tasik Chini. Proceedings of the National Seminar on Environmental Management. Universiti Kebangsaan Malaysia: Bangi, pp: 40-46.
15. World Health Organization (WHO), 1984. Guidelines for Drinking Water Quality: Health Criteria and other Supporting Information. Vol. 1. Geneva: World Health Organization.
16. Hammer, M.J. and K.A. MacKichan, 1981. Hydrology and quality of water resource. John Wiley and Sons: New York.
17. Laenen, A. and D.A. Dunnette, 1997. River Quality Dynamics and Restoration. Lewis Publishers: Boca Raton.

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 48-50, 2007
ISSN 1818-6769

Cytochrome C Affects The Viability and Fertility of Bull Semen

Zdrave Zahariev, Rossen Stefanov, Milko Sabev,
Kremena Miteva and Ivan Nikolov

Institute of Biology and Immunology of Reproduction,
Bulgarian Academy of Sciences, Sofia, Bulgaria

Abstract: This study was carried out to determine the effect of antioxidant cytochrome C, by supplement at concentration 1.5 mg ml^{-1} to Nagase-Niwa's diluent on motility, viability and fertility of bull spermatozoa. Cytochrome C improved significantly viability of spermatozoa of fresh semen and after deep freezing. Cytochrome C does not influence significantly on the viability of spermatozoa during deep freezing. The conception rate of cows after calving is increased with 8.3% and with 5.4% higher at non return ($p<0.05$).

Key words: Cytochrome C % spermatozoa % deep freezing % viability % fertility

INTRODUCTION

The investigations of number of authors revealed that the activity of enzymes systems is directly related to viability and fertilizing capacity of spermatozoa. The capacity of spermatozoa to perform independent movement is a characteristic physiological feature related to their biological function-to fertilize the ovule. This fact justifies the interest shown by many authors who attach great significance to the enzymes taking part in the processes of fertilization [1, 2].

An important reason for the decrease in the fertility during storage of semen is formation of peroxides in presence of oxygene radicals [3]. The deep freezing increases sensitivity to lipid peroxidation [4, 5].

A large number of antioxidants have been tested in an attempt to minimize the peroxidation [6]. Superoxide dismutase (SOD) and glutathione peroxidase-glutathione reductase-system which plays a protective role against damage-associated with oxygen metabolism [4]. Cytochrome C could also function as a potent antioxidant whereas is a role beyond its utility as an electron carrier [7]. Under normal physiological conditions, cytochrome C is attached to the outside of the inner mitochondrial membrane to function as an electron carrier-with a rise of Reactive Oxygene Species (ROS) levels [8]. With a rise of mitochondrial ROS levels, cytochrome C detaches from the inner mitochondrial membrane and is capable of oxidizing O_2 to form molecular oxygene [9].

The antioxidants-SOD, cytochrome C, catalase, glutathione peroxidase increase both the survival and acrosome integrity of spermatozoa during storage at 5 and 25°C [6]. Therefore it is important to determine whether the improved viability of spermatozoa would be reflected on the fertility. In the present study we examined benefits of cytochrome C-water soluble antioxidant for the viability and the fertility of bovine spermatozoa after the deep freezing.

MATERIALS AND METHODS

Collection, dilution and deep freezing of semen: Bull semen from 2 Holstein Frisian bulls was used for Artificial Insemination (AI). The semen was collected by artificial vagina and its semen quality was assessed and accepted as a donator, every ejaculate had full quality: volume >5.0 ml, macroscopic good visual mass, activity, sperm concentration > 0.8×10^9 and progressive sperm motility >70%. For the freezing, fresh bull semen was diluted in Nagase-Niwa's diluent 1:3 (semen:diluent) at 30°C. After the equilibration for 4 h at 3°C the semen was frozen according to the technology of Nagase-Niwa.

Thawing of deep frozen semen: The semen was thawed at 39°C as follows: sodium citrate -2.8 g, dest. water -100 ml. The diluent was divided into two equal parts: without and with 1.5 mg ml^{-1} cytochrome C (Fluka, Switzerland). The pH-values of medium were 7.5 and osmolarity 201 m Osm $kg\ mol\ 1^{-10}$ (Cryoscopic osmometer of medium Osmomat-30, Gonotec, Berlin, Germany). The frozen semen (cytochrome C is supplemented in the medium during the freezing) in pellet at 0.15 ml, containing $10\text{-}15\times10^6$

Corresponding Author: Prof. Dr. Zdrave Zahariev, Institute of Biology and Immunology of Reproduction, 73, Blvd. Tzarigradsko shosse, 1113 Sofia, Bulgaria

Table 1: Effect of cytochrome C on the viability and fertility of bull spermatozoa (Experiment 1)

	Motility of the spermatozoa (%)			Viability of the spermatozoa at 39°C (min)				Fertility of the spermatozoa		
								Without CHC	With 1.5 mg mlG^1 CHC	
		With			With	Distinction				
	Without	1.5 mg mlG^1	Distinc-	Without	1.5 mg mlG^1			Cows calved/total	Cows calved/total	
Variants	CHC	CHC	tion	CHC	CHC	min	%	insemination	insemination	Distinction
Variant A	76.0±8.1	76.0±8.1	-	491±12.92**	654±22.40**	+163	33.2	-	-	-
Variant B	35.5±0.8	37.1±1.2	+1.6	311±12.56	357±23.10	+46	14.8	-	-	-
Variant C	35.5±0.8*	41.0±1.8*	+5.5	311±12.56**	483±27.40**	+172	55.3	64/144(44.4%)	41/77(53.2%)	+8.3%

* p<0.01 ** p<0.001, Variant A - Fresh semen A^1 - (Cytochrome C is supplemented to the fresh semen), Variant B - Frozen semen B ^{1}- (Cytochrome C is supplemented to the medium during the freezing), Variant C - After the deep freezing of the semen C^1 - (CytochromeC is supplemented to the medium for the thawing), Abbreviation-CHC - Cytochrome C

Table 2: Effect of cytochrome C on the viability and fertility of bull spermatozoa after the deep freezing (Experiment 2)

	Variants				
			Distinction		
Indicators	Without CHC	With 1.5 mg mlG^1 CHC	min	%	p
Motility of the spermatozoa (%)	36.0±1.2	42.0±1.5	-	+6	= 0.050
Viability of the spermatozoa at 39°C (min)	352±2.8	516±3.1	+164	+46.6	= 0.001
Fertility of the spermatozoa (NR)	723^a/1203^b	738/1127 (65.5%)	-	+5.4	= 0.050

[a] - cows in pregnancy, [b] - artificial inseminated cows, Abbreviation: CHC - Cytochrome C

progressive motile sperm was thawed at 39°C in 0.5 ml of the diluent. For the investigations of the viability of thawed semen, the pellet at 0.15 ml was placed in 0.5 ml medium without and with cytochrome C (cytochrome C is supplemented in the thawing medium). All aliquots were incubated at 39°C and spermatozoa were assessed for motility.

Fertility trials: The artificial insemination of cows (Holstein Frisian) was performed by bimanual method. Estrous onset was detected and noted by an experienced person, who carried out continuous observations through watching behavior and clinical and gynecological symptoms characteristic for the estrous in females of this species [10]. The cows were inseminated with deep frozen semen in the pellet from 0.15 ml in volume with 10-15×10^6 progressively motile spermatozoa. The semen was thawed at 39°C in 0.5 ml of diluent without cytochrome C (control sample) and in the sample containing 1.5 mg mlG^1 cytochrome C (cytochrome C is supplemented in the thawing medium). The fertility rate was determined at first artificial insemination after calving (experiment 1) and at Non Return (NR) 60 days after insemination (experiment 2).

RESULTS AND DISCUSSION

Experiment 1: Data analysis showed that cytochrome C significantly influenced on the viability of spermatozoa during incubation at 39°C in fresh semen and after deep freezing. Cytochrome C does not influence significantly on the viability of the spermatozoa during deep freezing (Table 1). As it is seen from Table 1 in fresh semen and after deep freezing there are considerable differences in the viability of spermatozoa. In fresh semen in medium containing cytochrome C -1.5 mg mlG^1 the viability of the spermatozoa is higher with 33.2%, compared to the control samples (p<0.001) and in the semen after deep freezing is respectively with 55.3% (p<0.001).

The conception rate of cows after insemination without and with 1.5 mg mlG^1 is presented at Table 1. The fertility is increased with 8.3%.

Experiment 2: In the medium containing cytochrome C, the motility of spermatozoa after deep freezing is with 6% higher (p<0.05) and the viability is higher with 46.6% (p<0.001), compared to the control samples (Table 2).

The conception rate of cows NR after insemination without and with 1.5 mg mlG^1 is presented at Table 2.

In experiment 2, the fertility is increased with 5.4% ($p<0.05$).

A large number of antioxidants have been tested in an attempt to minimize peroxidation [3, 6, 11, 12]. In the present study we examined the benefits cytochrome C for the fresh bull semen and after deep freezing. There is no significant effect of C when it is at supplement during deep freezing of the spermatozoa. It was important to determine whether the improved viability of spermatozoa would be reflected on the fertility. The ability of cytochrome C to improve the fertilizing capacity of spermatozoa *in vivo* was examined in experiment 1 and 2.The fertilization rates were better at the insemination with semen extended with diluent containing cytochrome C, than in its absence after deep freezing. The reduction in fertilizing capacity of spermatozoa after deep freezing may reflect either changes in the nature of the motility the cells, or the changes in their membranes. The main beneficial effects of cytochrome C may be associated with sperm ageing [13]. In addition cytochrome C function as a potent antioxidant that scavenges ROS in the mitochondria. The presence of high levels of cytoplasmic GSH (gluthatione) maintain Cytochrome C in an inactive and it has been suggested that cytochrome C will only induced programmed cell death if it is present in the cytoplasm in the oxidized state [14]. Indeed, the redox status of cytochrome C and thus its structure can be altered by the presence of ROS and reduced gluthatione [15]. Cytochrome C improved the survival of spermatozoa in liquid storage at both 5 and 25°C [6].

From the results of present study we can recommend the supplement of different antioxidants to the diluents at storage and after the deep freezing of the sperm for improving the quality of the semen used in artificial insemination.

REFERENCES

1. Gavella, M. and P. Cvitkovic, 1985. Semen LDH-X deficiency and male infertility. Arch. Androl., 15: 173-176.
2. Zahariev, Z., H. Al-Khanak, M. Subev, I. Nikolov and R. Stefanov, 2001. Biological role of some enzymes and enzyme's systems in semen. Andrologiia, 2: 13-21.
3. Aitken, R.Y., 1995. Free radicals, lipid peroxidation and sperm function. Reprod Fertil Dev., 7: 659-668.
4. Alvarez, J.G. and B.T. Storey, 1992. Evidence for increased lipid peroxidative damage and loss superoxide dismutase activity as a mode of sublethal cryodamage to human sperm during cryopreservation. J. Androl., 13: 232-241.
5. Trinchero, G.D., M.A. Affranchino, L.M. Schang and M.T. Beconi, 1990. Antioxidant effect of bovine spermatozoa on lipid peroxidation. Com. Biol., 8: 339-350.
6. Maxwell, W.M. and T. Stojanov, 1996. Liquid storage of ram semen in the absence or presence of some antioxidants. Reprod Fertil Dev., 8: 1013-1020.
7. Korshunov, S.S., B.F. Krasnilov, M.O. Preventrel and V.P. Skulachev, 1999. The antioxidant function of cytochrome C. FEBS Lett., 462: 192-198.
8. Skulachev, V.P., 1998. Cytochrome C in the apoptic and antioxidant cascadesc. FEBS Lett., 423: 275-280.
9. Skulachev, V.P., 1995. Non phosphorylating respiration at the mechanism preventing the formation of reactive form of oxygen. Mol. Biol., 29: 1199-1209.
10. Hafez, B. and E.S.E. Hafez, 2000. Reproductive behavior in: Hafez ESE and Hafez B, editors. Reproduction in Farm Animals, 7th Edn. Baltimor, M.D., Lipincott Willams and Wilkins, pp: 293-306.
11. Funahashi, H., 2005. Effect of beta-mercaptoethanol during *in vitro* fertilization procedures on sperm penetration into porcine oocytes and the early development *in vitro*. Reproductia, 130: 889-898.
12. Vishwanath, R. and P. Shannon, 1997. Do sperm cells age? A review of the physiological changes in sperm during storage at ambient temperature. Reprod. Fertil. Dev., 9: 321-332.
13. Watson, P.F., 2006. Recent developments and concepts in the preservation of spermatozoa and assessment of their post-thawing function. Reprod Fertil Dev., 13: 871-891.
14. Ueda, S., H. Nahamura, H. Masutani, T. Sasada, S. Yonehara, Y. Yamaoka and J. Yoadai, 1998. Redox-activity regulatory role of thioredoxin and cytochrome C. J. Immunol., 161: 6689-6695.
15. Chen, Q., M. Crosby and A. Almasan, 2003. Redox regulation of apoptosis before and after cytochrome C release. Korean J. Biol. Sci., 7: 1-9.

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 51-61, 2007
ISSN 1818-6769

The Effect of Using Urban Treated and Untreated Effluents on Soil and Agricultural Crops Pollution in Syria (Damascus-Ghouta)

Omer Jouzdan, F. Fares and G. Abedalgawad

Division of Soil and Water Uses, The Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD), Damascus, Syria

Abstract: Polluted soil and irrigation water are the most important sources of pollution of agricultural products, with heavy metals mainly field crops and vegetables. A lysimeter experiment cultivated with field crops and vegetables was carried out to study the effect of irrigation with treated urban effluents resulting from Adra plant which receives waste water from Damascus. The heavy metals (As, Cd, Cr and Pb) increased in fruits and leaves of the studied vegetables (Eggplant and Lettuce), as well as in the grain and straw of studied field crops (Wheat and Corn), grown in the concrete lysimeters of 2x1x1m. The pollution caused by irrigation with urban treated effluents was compared with that caused by irrigation with both ground and urban untreated effluents. During the course of the study which extended over two years with two successive seasons per year were grown of the selected four crops, showed the following most important results: A moderate increase of the soil content of heavy metals (As, Cd, Cr and Pb). An increase of the heavy metals contents in the crops components (seeds, fruits and leaves), according to the water quality: (groundwater, urban untreated effluents and urban treated effluents). However, with the exception of Cd, the content of As, Cr and Pb in the crops, remained within the acceptable natural range.

Key words: Urban treated and untreated effluents % urban under ground water % heavy metals % soil and plant tissues

INTRODUCTION

Human and animal wastes had been used to improve agricultural production in the past in several countries. The use of such wastes goes back to 5000 years ago. The use of solid and effluent wastes in the past intended to reduce polluting river water and to economize water use. In Great Britain for example, there is a slang known as rain water for rivers and urban waste water for the soil [1].

In the Arab world Egypt was the first country who used waste effluents and solid wastes in agriculture. It started in 1911 where they used effluent as irrigation water and solid waste as fertilizer in the yellow mountain agricultural area near Cairo.

In Syria, Damascus city waste water drained intro Brada River for a long period of time. The mixture was used for irrigating several crops in Damascus Ghouta without restrictions until 1996. In Aleppo (south Aleppo plain) Homs and Hama provinces, the untreated waste water was used in irrigation for several years back.

Due to the population increase in Damascus province, water shortage started to increase not only for irrigation but also for domestic supply. Hence it became a necessity to look for other sources of irrigation water to reduce the pressure on the fresh water. The urban untreated waste effluents in Damascus province was estimated to be 610 $m.m^3$/year in 1993 and jumped to 740 $m.m^3$/year in 2002 [2, 3].

The sewage wastewater in Damascus province consists of sewage effluents, industrial effluents etc., Hamad *et al*. [4] has studied Damascus urban wastewater and found it containing several heavy metals with various concentrations. Chromium has the highest concentration among the studied heavy metals. Several types of pathogenic microbes such as Salmonella, Fecal coliform etc.... Several others [4, 5-9] found similar results. These pathogens and heavy metals can affect both human and animal health [4, 10, 11]. These effects depend on the loads of the waste effluents with different components, which depends "in turn" on local communities' habits and

Corresponding Author: Dr. G. Abedalgawad, Division of Soil and Water Uses, The Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD), Damascus, Syria

the components of different sources of the urban waste effluents. The urban waste effluents contents will affect the crop components irrigated with these effluents, will depend on the type of crop, the soil and the growing season, their rates of accumulation in soils and their movements out of the root zone and plant uptake rates [12-15].

The health and environmental hazards of heavy metals are thoroughly studied by researchers [7, 16-20]. But there is no research work done in Syria about the use of treated urban effluents in irrigation. This study has been carried out to disseminate the safe use of treated urban effluents for irrigating agricultural field crops and Horticultural crops and to provide information to the local communities and decision makers about its proper use. The use of urban treated waste effluents in Syria was studied for the first time in 1998 [21]. This treated effluents used for irrigation of 18000 ha in Damascus Ghouta by local communities. The major objective of this paper is to study the expected effects on the use of such effluents on the quality of local renewable natural resources such as soil, water, crops etc., through the following:

- Chemical characterization of this kind of water such as soluble ions, pH, EC and heavy metals.
- Accumulation of some heavy metals in soils.
- Crop content of heavy metals such as eggplant, wheat, corn and lettuce tissues.
- Monitoring of urban treated waste effluents and their load of chemicals and comparing them with ground water as a control.

MATERIALS AND METHODS

Materials:

A-soils: The soil used for this study is classified by USDA, 1997 soils taxonomy, as calcid soil, collected from Nashabia area 20 km south of Damascus city center [22].

The soils were placed in the lysimeters according to their natural layers in the field. Table 1 shows some chemical properties of the used soil layers.

The soil is clay in texture with average bulk density of 1.11 g cm^{-3} and with real density of 2.66 g cm^{-3}. The saturation moisture % is 58% while the field capacity is 27.5% with wilting points of 8.8%. The available moisture is 18.7% and having saturated hydraulic conductivity of 161.4×10^{-3} cm h^{-1}. The soil is classified as slow infiltration rate soil. Table 1 shows some chemical properties of this soil which classify the soils as alkaline with pH 8.5. Having EC1:5 of 0.2 dS m^{-1}, with average CEC of 17.79 $cmol_c$ kg^{-1}. The soil has an average $CaCO_3$ of 59.80 and 1.29% organic matter. The soil is low in available phosphorus, potassium and nitrogen.

The total content of some heavy metals in the studied soils are presented in Table 2.

Analysis of these heavy metals is found to be within their natural contents of non polluted agricultural soils [5, 16, 23, 24].

B-irrigation water: Three types of irrigation water were used in this study:

- Under ground water with static water level at 120 m depth in the experimental site (T1).
- Treated waste water from Adra treatment plant 25 km east of Damascus city (T2)
- Untreated waste water before entering Adra treatment plant (T3).

From Table 3 we can conclude, that under groundwater contains low potassium values with an average of 0.21 $mmol_c$ l^{-1} and with calcium, magnesium and nitrate contents 5.14 and 3.56 $mmol_c$ l^{-1} and 10.0 mg l^{-1}, respectively. The presence of nitrate is due to heavy rate of nitrogenous fertilizers application to the soil in the last four decades. Nitrate concentrations are higher than in the urban treated effluents and urban waste

Table 1: Chemical properties of soil layers in lysimeters-before cultivation 1998

	pH (1:25)		E.C	Soluble Ions ($mmol_c$ kg^{-1})*											Effective			
Depth	----------		1:5	----------									Av.P	T.N	$CaCO_3$	$CaCO_3$	O.M	C.E.C
(cm)	(H_2O)	(KCl)	dS m^{-1}	Cl^-	$CO_3^=$	HCO_3^-	$SO_4^=$	Na^+	K^+	Ca^{++}	Mg^{++}	SAR	mg kg^{-1}	%	%	%	%	*$cmol_c$ kg^{-1}
0-15	8.50	7.90	0.19	0.51	0	0.80	0.09	0.17	0.01	0.60	0.50	0.23	0.60	0.05	18.13	62.00	1.35	19.35
16-30	8.50	7.90	0.19	0.70	0	0.95	0.09	0.20	0.02	0.95	0.45	0.24	0.40	0.05	19.00	62.00	1.31	17.39
31-45	8.40	7.90	0.20	0.79	0	1.00	0.09	0.30	0.02	1.10	0.35	0.35	1.36	0.04	18.50	55.00	1.30	17.39
46-60	8.30	7.90	0.20	0.90	0	0.85	0.09	0.26	0.02	1.15	0.35	0.30	0.40	0.04	19.38	59.00	1.30	17.39
61-75	8.50	7.90	0.20	1.10	0	0.85	0.09	0.34	0.02	1.15	0.50	0.37	2.20	0.04	19.00	61.00	1.21	17.39
Average	8.44	7.90	0.20	0.8	0	0.89	0.09	0.25	0.02	0.99	0.43	0.30	0.99	0.04	18.80	59.80	1.29	17.78

*$cmol_c$ kg^{-1} = meq/100 g soil

Table 2: The total content of some heavy metals in the studied soil before cultivation

	(mg kgG¹)			
Depth (cm)	As	Cd	Cr	Pb
0-15	2.40	0.07	10.5	7.9
16-30	1.9	0.06	11.3	7.6
31-45	1.50	0.05	11.3	6.8
46-60	1.20	0.06	14.1	5.3
61-75	0.90	0.07	16.4	2.6
Average	1.58	0.06	13.0	6.0

Table 3: Some important irrigation water parameters

Type of irrigation water and effluents	pH	E.C dS mG¹	Ions ($mmol_c$ lG¹) ClG	HCO_3^-	SO_4G	Na^+	K^+	Ca^{++}	Mg^{++}	(mg lG¹) NO_3G	$NH4^+$	PO_4G	B	BOD
Underground water	7.38	0.86	3.15	6.33	0.13	0.68	0.21	5.14	3.56	10.06	0.05	0.48	0.21	15
Treated	7.40	0.91	3.27	4.96	0.15	1.49	1.28	3.26	2.19	0.40	1.30	8.73	0.63	55
Untreated	7.08	1.07	3.45	6.10	0.17	1.53	1.32	3.95	3.03	0.26	6.41	9.95	0.92	122.5

(*) Every value is an average of 48 analyses (4 seasons× 4 periods)×3 replicates

untreated effluents as shown in Table 3. Ammonium and PO_4 = concentrations are higher in untreated effluents, compared with underground water and urban treated effluents. These values are verified by statistical analysis of the untreated and treated effluents, as example for NH_4^+ (6.41, 1.30 mg lG¹), PO_4 = (9.95 and 8.73) mg lG¹, for Boron (0.93 and 0.63) mg lG¹ for untreated and treated waste respectively, in addition both untreated and treated effluents having high values of BOD 122.5 and 55 mg lG¹ respectively, while the underground water has a value of 15 mg lG¹.

C-irrigated crops: Two summer crops and two winter crops were used in this study:

- Summer crops: The summer crops cultivated in the lysimeters were Eggplants (*Solanum melongena*) local variety and corn (*Zea mayes*) Ghouta variety.

- Winter crops: were wheat (*Triticum durum*) Sham 5 variety and Lettuce (*Lactuca sative*) local variety.

Methods of measurements and analysis:

Soil water and effluents chemical analysis: pH was measured in soil water ratio of (1:2.5), effluents and water samples with pH meter (Beckman model). The EC of soil extract was carried out in soil water ratios of 1:5 with conductivity meter (Hach Instrument Company) as well as the urban treated and untreated effluents samples. The soluble ions in soil extracts and in irrigation water as follows. Chloride ion by titration with $AgNO_3$, SO_4 = by turbidity [25], CO_3 = and HCO_3 by titration using H_2SO_4. Sodium and Potassium measured using Flame photometer (Jen way PFp7. UK).

The $CaCO_3$, BOD, B and effective $CaCO_3$ were determined according to the methods in the Methods of soil analysis [26]. The available P was determined according to Olsen and Sommers [27], total Nitrogen by kjeldahl method [28], organic matter according to Methods of soil analysis [29], CEC (Cation exchange capacity) by Na-Acetate method according to Rhoades and Polemio [30]. The nitrate content in irrigation water was determined by phenol disulfonic (C_6 H_6 O_7 S_2) [31]. Ammonium ion determined by Endol blue method [32].

Heavy metals analysis: The total content of Pb, Cr, Cd and As in irrigation water (underground water, urban treated and urban untreated effluents) were analyzed by Atomic absorption GPC 932 AA [33]. The total heavy metals of Pb, Cr, Cd and As in soils were taken at each 15 cm soil depth. Soil samples were air dried, grinded and then passed through 0.5 mm sieve. One gram of the soil sample from each soil depth heated to 800°C for 2 h then the sample digested with 5 ml of 65% HNO_3 and 19 ml of 38% HCl [34] and heated in water bath until almost complete dryness, then the suspension filtered and diluted to 100 ml of double distilled water.

The total heavy metals in plant tissues at the end of the each experiment were determined. Plant tissues were washed with tap water then with double distilled water, dried in an oven at 50°C for 48 h, grinded to very fine

Table 4: Irrigation water treatments of this study

Treatments	Type of irrigation water
T1	Underground water as control
T2	Urban treated waste effluents
T3	Urban untreated waste effluents

Table 5: Average concentrations of Pb, Cr, Cd and As in mg l^{-1} in the irrigation water

	Type of heavy contents in mg l^{-1}			
Type of irrigation water	Pb	Cr	Cd	As
Underground water	0.5 C	0.02 B	0.016 A	0.003 B
urban treated effluents	2.7 B	0.03 AB	0.014 A	0.018 AB
Urban untreated effluents	4.9 A	0.04 A	0.015 A	0.031 A
The upper limit concentrations for irrigation	5.0	0.10	0.01	1.10
$LSD_{0.05}$	0.782	0.017	0.012	0.023

materials, then one gram sample, dry ached at 1000°C for one hour, the dry ached materials were digested with 10 ml concentrated HNO_3 acid with slow heating rate in water bath. The samples then filtered and diluted to 100 ml with distilled water in volumetric flask. The heavy metals (Pb, Cr, Cd and As) were determined with Atomic Absorption GPC 932 AA.

Experimental design: The study was designed according to Complete Randomized Block Design. The experiments consisted of 3 water treatments Table 4 with three replicates.

The number of experimental blocks: Two sets of Lysimeters (18 Lysimeters each) were used in the study. The first set was planted by eggplant and wheat and the second by corn and lettuce. Each set of lysimeters contained 3 replicates with 3 irrigation water treatments and 2 crops (3×3×2 = 18 lysimeters) the area each lysimeter was 2×1 = 2 m^2 and the length 1 m.

RESULTS AND DISCUSSIONS

Heavy metals monitoring in this study:

In irrigation water and effluents: Table 5 shows the average heavy metals contents of different irrigation waters (underground water, urban treated and urban untreated effluents). The heavy metals were Pb, Cr, Cd and As. These average concentrations of these heavy metals are presented in Table 5 during course of this study (1998-2000).

Each value in Table 5 is the average of 48 analyses. The statistical analysis ensures the save use of the treated urban effluents for irrigation concerning heavy metals, noting that these data are the results of continuous analysis of these irrigation water and effluents for two years. These values are lower than the upper limits which are listed by many researchers [6, 7, 17, 20, 35-37].

It is worth to note the followings from Table 5: 1) relatively high content of lead in urban untreated effluents and urban treated effluents (4.9 and 2.7 mg l^{-1}), respectively, 2) relatively high content of Cr (0.04 and 0.03 mg l^{-1}) in urban untreated effluents and urban treated effluents respectively, compared with ground water (0.02 mg l^{-1}). Cadmium concentrations are within the upper limit concentration of WHO standards of all water treatments. Arsenic is below the upper limit of WHO standard. Chromium values for underground, treated urban effluents and untreated urban effluents are below the upper limit of WHO standards. Cadmium values are as an important issue for the water and effluents treatments as well as farmers. Therefore, it should be taken into consideration. Farmers should not use urban untreated effluents because its concentration exceeds the upper limit [20]; moreover, it contains pathogenic microbes.

Monitoring heavy metals in soil: Table 6 shows total heavy metals analysis at various soil depths in Lysimeters after two years of eggplants, wheat, eggplant cultivations irrigated with three types of irrigation water and effluents.

From Table 6 we can note that total average concentrations of lead at the end growing seasons in the soils cultivated with Eggplants and irrigated with the 3 water quality treatments have the following order T3>T2>T1. Lead accumulated in the soil depth 0-45 cm for all treatments. The accumulated of Pb in the irrigated soils is far below the upper limit (200 mg kg^{-1}). Chromium accumulation had the same trend. Cadmium had the same concentrations in all soils irrigated with T1, T2 and T3 treatments. Cadmium concentration is still within the natural range in unpolluted soil. Cadmium moved down to the depth of 46-75 cm from the upper soil depths 0-30. This phenomenon is in the agreements with the finding of Hille *et al*. [38], Abo Rous and Samir [39] and Abdelgawad [40]. Arsenic accumulated at the surface of the soil and in the upper 0-30 cm soil depth is higher than the lower depths. Arsenic concentration is still within the range listed in the literature for unpolluted soil.

Table 7 shows that the total concentrations of Pb,Cd, Cr and As in soils at various soil depth in lettuce-corn lysimeters irrigated with treatments T1,T2 and T3 for the

Table 6: Total concentrations of Pb, Cr, Cd and As at various soil depths after two years of cultivations (1998-2000) with wheat, eggplant

Water treatment	Soil depth cm	Heavy metal concentrations in mg kgG¹ Pb	Cr	Cd	As
T1	0-15	9.51	13.48	0.086	2.80
	16-30	10.85	12.58	0.089	2.55
	31-45	11.02	11.28	0.173	1.16
	46-60	8.68	11.68	0.086	1.25
	61-75	7.54	12.78	0.216	0.74
Average		9.52 C	12.36	0.13	1.70
T2	0-15	17.61	12.38	0.085	2.73
	16-30	17.33	12.24	0.149	2.12
	31-45	15.85	12.78	0.021	1.48
	46-60	13.03	12.64	0.149	0.62
	61-75	12.63	13.06	0.146	0.45
Average		15.29 B	12.62	0.110	1.48
T3	0-15	29.86	13.76	0.100	3.01
	16-30	29.64	12.62	0.136	2.21
	31-45	24.50	13.02	0.073	1.48
	46-60	24.00	12.69	0.118	0.99
	61-75	21.35	12.56	0.123	0.56
Average		25.87 A	12.93 A	0.110	1.65
Upper limit range		2.0-200	10-150	2-0.01	1.1-80
$LSD_{0.05}$		1.963	NS	NS	NS

T1 = Underground water, T2 = Urban treated effluents, T3 = Urban untreated effluents

Table 7: Total concentrations of Pb, Cr, Cd and As at various soil depths after two years of cultivation (1998-2001) with lettuce and corn

Water treatment	Soil depth cm	Heavy metal concentrations in mg kgG¹ Pb	Cr	Cd	As
T1	0-15	9.52	13.47	0.108	1.69
	16-30	11.42	15.20	0.112	1.62
	31-45	7.52	17.00	0.104	1.58
	46-60	8.38	17.93	0.096	1.56
	61-75	6.56	15.40	0.080	1.45
Average		8.68 C	15.80	0.100	1.58
T2	0-15	14.91	14.44	0.096	1.82
	16-30	14.33	15.31	0.077	1.74
	31-45	12.91	16.44	0.092	1.67
	46-60	10.44	15.75	0.08	1.54
	61-75	7.81	16.56	0.097	1.88
Average		12.08 B	15.70	0.090	1.73
T3	0-15	24.03	16.23	0.096	2.07
	16-30	22.12	17.26	0.104	2.00
	31-45	18.82	16.74	0.108	1.72
	46-60	14.85	18.46	0.098	1.5
	61-75	11.58	13.31	0.094	1.26
Average		18.28 A	16.40	0.100	1.72
Upper limit range		2.0-200	10-150	0.01-2	1.1-80
$LSD_{0.05}$		1.892	NS	NS	NS

periods (1998-2000), had the order T3>T2>T1 treatments and values of 18.28, 12.08 and 8.68 mg kgG¹, respectively. Lead accumulated in the upper 0-45 cm of the soil. The average values of lead concentration in the soils irrigated with T1, T2 and T3 are lower than the upper limit of lead concentration which is 200 mg kgG¹ according to Who [20]. Chromium accumulation in the soils irrigated with T3 treatments was higher than T1 and T2. Cadmium concentrations in soils irrigated with the three treatments were similar and no clear pattern of its accumulation in soils for T2 and T3 treatments but the pattern was clear in the soil irrigated with T1. Arsenic accumulation in soils irrigated with T3 and T2 treatments were higher than T1 water treatment. Arsenic accumulated in the upper soil 0-45 cm depth. Both Arsenic and cadmium concentrations were higher than the lower polluted soil limits mentioned in literature.

The statistical analysis showed significant differences at 5% level of Pb concentration between irrigation water treatments Table 7. The differences between irrigation treatments were not significant for Cr, Cd and As. The bioavailability of these heavy metals depend on their concentrations in soil solution, which are related to their total concentrations in the solid phases, pH, redox potentials, CEC and other soil properties [41]. For example, the bioavailability of Cr depends on its Oxidation state where Cr^{+6} is very toxic to the living organisms while Cr^{+3} is less toxic. The heavy metals which are inter in organometallic complexes in soils decrease their bioavailability to living organism in soils as well as plant roots, they become slowly available in the soil solution. The bioavailability depends upon biological activities and chemical properties of Rizosphere of plant roots system such as pH, as well as the selectivity of the crop type to absorb certain heavy metals from others.

Plant tissues heavy metals content: In this study, it was found that all the irrigation water treatments caused accumulation of the studied heavy metals in the studied plants tissues. These accumulations in plant tissues increased with growth stages and growing seasons which are in agreement with [3, 5, 42, 43]. Tables 8-11 show the concentration of Pb, Cr, Cd and As in Eggplant Fruits and green materials, what stem and leaves, corn grain and straw and Lettuce leaves respectively. All values are the average of two growing seasons for the three irrigation treatments. Tables 8 & 9 show that Pb, Cr, Cd and As concentrations in green materials of Eggplant and corn are greater than fruits and grains in all treatments including

Table 8: Average total contents of Pb, Cr, Cd and As in eggplant on oven dry weight bases in mg kg^{-1} for different irrigation water treatments

Element	Pb		Cr		Cd		AS	
Normal range	3-20		0.5-2		0.02-0.2		0.02-10	
Toxic level	30-300		5-30		5-30		>10	
Part	Fruits	Green	Fruits	Green	Fruits	Green	Fruits	Green
T1	1.69B	1.86 B	0.95B	1.20C	0.14B	0.18B	0.01B	0.02B
T2	7.56 A	8.33 A	1.95A	4.40B	0.29A	0.31A	0.04A	0.06A
T3	7.75 A	8.28 A	2.01A	4.79A	0.32A	0.34A	0.05A	0.07A
$LSD_{0.05}$	0.578	0.398	0.152	0.264	0.081	0.081	0.015	0.018

Table 9: Average total contents of Pb, Cr, Cd and As in wheat on oven dry weight bases in mg kg^{-1} for different irrigation water treatments

Element	Pb		Cr		Cd		AS	
Normal range	3-20		0.5-2		0.02-0.2		0.02-10	
Toxic level	30-300		5-30		5-30		>10	
Part	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T1	3.44 C	0.29C	0.17C	0.15C	0.28B	0.26B	0.11B	0.08B
T2	5.22B	2.13B	0.57B	0.40B	0.34A	0.29AB	0.18A	0.15A
T3	5.81A	3.09A	0.70A	0.58A	0.35A	0.35A	0.19A	0.17A
$LSD_{0.05}$	0.471	0.161	0.126	0.055	0.086	0.098	0.059	0.04

Table 10: Average total contents of Pb, Cr, Cd and As in corn on oven dry weight bases in mg kg^{-1} for different irrigation water treatments

Element	Pb		Cr		Cd		AS	
Normal range	3-20		0.5-2		0.02-0.2		0.02-10	
Toxic level	30-300		5-30		5-30		>10	
Part	Fruits	Green	Fruits	Green	Fruits	Green	Fruits	Green
T1	2.23C	4.33C	0.12B	0.38B	0.26	0.29	0.00B	0.01C
T2	8.92B	10.68B	0.91A	1.14A	0.28	0.36	0.02A	0.45B
T3	9.57A	12.05A	0.97A	1.24	0.28	0.38	0.02	0.48A
$LSD_{0.05}$	0.263	0.149	0.089	0.154	NS	NS	0.003	0.006

Table 11: Average total contents of Pb, Cr, Cd and As in lettuce on oven dry weight bases in mg kg^{-1} for different irrigation water treatments

Element	Pb	Cr	Cd	AS
Normal range	3-20	0.5-2	0.02-0.2	0.02-10
Toxic level	30-300	5-30	5-30	>10
Part	Leaves	Leaves	Leaves	Leaves
T1	1.83B	0.43B	0.22B	0.17B
T2	3.67A	0.67A	0.34A	0.45A
T3	3.93A	0.75A	0.38A	0.48A
$LSD_{0.05}$	0.261	0.089	0.062	0.054

the control. The concentrations of these metals increase in the order of T3 >T2>T1 water treatments. For As and Pb their concentrations are found to be within their natural ranges in plant tissues for all crops cultivated in this study, for the three irrigation water treatments. Cadmium concentrations in all studied plant tissues are higher than the normal concentration in natural plant tissues, (0.02-0.2 mg kg^{-1}). Chromium concentrations in plant tissues are within its natural conditions except the vegetative parts of Eggplant for treatments T3 and T2 which are higher than normal.

The statistical analysis presented in Tables 8-11 show significant differences in their concentrations in the studied plants for all studied elements between irrigation water treatments except for Cd in corn. In all cases the concentration is the lowest in the treatment irrigation with underground water. That is because the low concentration of these elements in the underground water which is attributed to their adsorption on soil particles during the infiltration process. This means that the underground water can be used safely for irrigation concerning the studied heavy metal.

Heavy metals balance: Table 12 shows the soil water balance for the Lysimeters cultivated with Eggplants, Wheat, Lettuce and Corn. The total water used by crops

Table 12: Water balance for crops used in each study at 80% soil field capacity

Crop	Ave rage initial soil moisture (Si) m^3 ha^{-1}	Average irrigation water added (I) m^3 ha^{-1}	Average amount of water used at the end of growing season to leach the soil (L) m^3 ha^{-1}	Average rainfall (P) m^3 ha^{-1}	Amount of leached water (D) m^3 ha^{-1}	Average final soil moisture (S_f) m^3 ha^{-1}	Average total water used m^3 ha^{-1}	Number of irrigation Norms	Length of growing season (day)
Eggplants (Summer)	1575	9180	1000	0	135	2475	9145	15	144
Wheat (Winter)	1305	4227	1400	695	148	2619	4860	9	182
Corn (Summer)	1476	4875	1400	0	150	2475	5126	8	90
Lettuce (Winter)	1458	3680	1000	695	140	2610	4083	7	146

Table 13: Average balance for Pb, Cr, Cd and As by calculation and analysis in mg/2 m^3 of soils in Lysimeters cultivated with (Wheat and eggplant for two year)

Water treatment	Initial soil contents	Amount added with irrigation water and the added leached water	Amount absorbed by plant tissues	Amount in the leached water	The amount accumulated in soils by calculation	The amount accumulated in soils as determined by analysis	% of differences
AS							
T1	3157	18.97	0.16	0.6	3175	3288	-3
T2		113.81	0.46	2.5	3268	2965	10
T3		196	0.6	3.7	3349	3287	2
Cd							
T1	119.9	101.16	1.26	1.1	219	230	-5
T2		88.52	2.62	1.7	204	234	-14
T3		94.84	3.13	1.8	210	223	-6
Cr							
T1	25974	126.46	8.21	2.60	26090	26279	-1
T2		189.68	26.19	3.30	26134	25180	+ 3
T3		252.91	30.40	3.60	26193	25800	+2
Pb							
T1	25974	126.46	8.21	2.60	26090	26279	-1
T2		189.68	26.19	3.30	26134	25180	+3
T3		252.91	30.40	3.60	26193	25800	+2

* Absorbed by Eggplants + wheat during two growing seasons in mg of plant tissues grown in Lysimeters, T1 = Underground water treatments, T2 = Urban treated effluents, T3 = Urban untreated effluents

were 9145, 4860, 5126, 4083 m^3 ha^{-1} for Eggplants, Wheat, Corn and Lettuce, respectively. The amount of water used for heavy metals balance calculation are the once that were added as irrigation water to Lysimeters.

This section was carried out to compare the analytical and calculated heavy metals concentrations. The balance carried out according to the following:

Soil basic concentrations before cultivation + the amount added with different types of irrigation water-(Amount taken up by plants + amount in the leachate + amount accumulated in the soils after two years of cultivation). This balance was carried out for each type of irrigation water. Tables 13 & 14 show this balance.

From Table 13 we note that the amount of As accumulated by calculation procedures were 3.175, 3.268 and 3.349 g /2 m^3 (the values were converted to g/2 m^2 by dividing the values in Table 13 and 14 by 1000) of soil for the Lysimeter cultivated by Eggplant and wheat for two growing season (wheat + Eggplant + wheat + Eggplant) for T1, T2 and T3 respectively but by analysis, they are 3.288, 2.965 and 3.287 g/2 m^3 soil (Lysimeter). The comparing shows a difference of 3, 10 and 2%. These differences are statistically acceptable. For Cd, the amounts accumulated in soils by calculations in the Lysimeters of (Wheat, Eggplant, Wheat, Eggplant) are 0.219, 0.204 and 0.210 g/2 m^3 soil while with analysis are 0.230, 0.234 and 0.223 g/2 m^3. The differences between them are 5, 14 and 6%, respectively for T1, T2 and T 3 respectively. The values of Cr are 26.09, 26.134 and 26.193 g/2 m^3 by calculations balance while by analysis the

Table 14: Average balance for Pb, Cr, Cd and As by calculation and analysis in mg/2 m^3 of soils in Lysimeters cultivated with (corn and lettuce for two year)

Water treatment	Initial soil contents	Amount added with irrigation water and the added leached water	Amount absorbed by plant tissues	Amount in the leached water	The amount accumulated in soils by calculation	The amount accumulated in soils as determined by analysis	% of differences
AS							
T1	3157	13.15	0.57	0.40	3169	2880	+ 10
T2		78.88	2.02	2.80	3231	3454	-6
T3		135.84	2.06	4.00	3287	3428	-4
Cd							
T1	119.90	70.11	4.98	0.90	184	190	-3
T2		61.35	8.10	1.30	172	176	-2
T3		65.73	7.14	1.80	177	183	-3
Cr							
T1	25974	87.64	7.94	2.60	26051	28914	-10
T2		131.46	30.70	4.40	26070	31232	-13
T3		175.28	29.17	4.90	26115	26810	-3
Pb							
T1	11988	2191.00	92.33	0.30	14086	15884	-13
T2		11831.00	284.20	1.70	23533	24110	-2
T3		21910.00	263.61	6.70	33628	36478	-8

*Absorbed by Lettuce and Corn during two growing seasons in mg of plant tissues grown in Lysimeters, T1 = Underground water treatments, T2 = Urban treated effluents, T3 = Urban untreated effluents

values are 26.279, 25.180 and 25.800 g/2 m^3. The differences are within 1, 3 and 2% above or below 100% which are really good for T1, T2 and T3. For Pb, the values by calculations are 15.132, 28.98 and 43.511 g/2 m^3 but by analysis, the values are 17.43, 30.500 and 51.606 g/2 m^3 for T1, T2 and T3 respectively. The differences between calculated and analysis are 13, 5 and 16% for T1, T2 and T3 respectively.

For the Lysimeters which were cultivated by Corn-Lettuce-Corn-Lettuce (Table 14) the concentrations of As by calculation for the three irrigation treatments were 3.169, 3.231 and 3.287 g/2 m^3 soil while by analysis are 2.880, 3.454 and 3.428 g/2 m^3 soil for the T1, T2 and T3 treatments respectively. In general they are comparable to each other. For Cd the concentrations by calculation were 0.184, 0.172 and 0.177 g/2 m^2 while by analysis are 0.190, 0.176 and 0.183 g/2 m^3 soil for T1, T2 and T3 respectively. The differences between the cultivated and the analyzed are within 3%. Chromium concentrations by calculations were 26.051, 26.070 and 26.115; while by analysis were 28.914, 31.232 and 26.810 g/2 m^3 soil for T1, T2 and T3 respectively. The difference range between 3 and 17%. Lead concentrations by calculations were 14.086, 23.533 and 33.628 and by analysis are 15.884, 24.110 and 36.478 g/2 m^3 soil for T1, T2 and T3 respectively. In general, there is a good agreement in heavy metals balance between calculated and analyzed.

CONCLUSIONS

The conclusions, which can be drawn from this study, are:

- The lead concentrations in soil increases with irrigation water and effluents added continuously. The accumulations mainly concentrated at the first soil depth (root zone 0-45 cm soil depth). This will cause in the future to more accumulation in this zone and bioavailability of heavy metals will increase with its rate of accumulation, this will lead to increase in its concentration in plant tissues. The rates of accumulations are in urban untreated effluents higher than the treated once respectively.
- There is similarity in the accumulations behaviors of As and Pb in soils. There are differences in their rates of accumulation in soils among different water and effluents used for irrigation in order of their increase, in their concentrations, urban waste untreated>urban treated waste effluents> underground water.
- There are no differences in the rate of Cd accumulations in soils due to different irrigation water and effluents used and are in order of untreated>treated>underground. This conclusion is confirmed with statistical analysis as shown before;

this is due to the quite similar concentrations of Cadmium in these irrigation waters and effluents.

- Chromium metal is progressly accumulated in soils; this accumulation is due to its concentrations in different water and effluents types in irrigation water. Its rate of accumulation in soils is higher in urban untreated effluents>urban treated effluents> underground water. Tanneries factories waste is the source of cr in the damascus urban waste effluents...
- The concentrations of Pb, Cr, Cd and As in plant tissues are higher in the treatments irrigated with urban untreated effluents>treated>ground water, in all plant tissues studied in this study, the concentration of Cd are higher than the upper normal concentrations in plant tissues as shown in Table 10 (0.2-0.02) mg kg^{-1}. For Pb, Cr and As are as well higher than the normal lower values of these metal concentrations in plant tissues respectively as shown in Table 10, but they are less than their toxic (values), for Cr its concentration is higher than the normal concentrations in plant tissues in Eggplant, (green cover) but not in fruits for both the treatments of treated and untreated waste effluents and still lower than its toxicity limits. Other elements (Pb, As) their concentrations are within the limit of normal concentrations in plant tissues.
- Based on the WHO, FAO, others international standards and Syrian standards of using treated waste effluents and the statistical analysis for monitoring such effluents in Damascus treatment plant, this effluent can be used safely for irrigation as far as the heavy metals studied but continuous monitoring of these elements and others is needed.
- It is important to follow up the concentrations of Cd, Pb in the treated effluents, because their concentrations are close to the upper limit standards of its irrigation water use especially Cd the sources of Cr, are tanneries factories effluents. These effluents should be well treated before drain it to the sewage drainage line.
- The heavy metals balance showed that the accumulations of heavy metals by calculations or analyses in soils are comparable to each other.
- In Syria, it is necessary to apply the law of the treated use in rigid way and rigid procedures in monitoring the qualities of treated effluents and its use. As well as long term effect should be studied.

ACKNOWLEDGMENTS

The Authors are grateful to professor Albert L. page from University of California at riverside department of environmental science and Dr Awadis Arsalan from ACSAD for reading the manuscript and providing us with their valuable comments

REFERENCES

1. Angelakis, A.N. and S. Spridakis, 1995. The status of water resources in Minion times: A preliminary study. Angelakis, A.N. and A. Issar, (Eds.). Diachronic climatic region. Springer-Verlag, Heideberg, Germany.
2. Abdelgawad Gilani, 1998. Treated waste water in the Arab region its sources and uses. Symposium on the treatment of waste water and its use. ACSAD. Damascus, Syria, pp: 1-20.
3. Jouzdan, Omar, 2002. Effect of irrigation with untreated sewage water, treated sewage water and ground water on soil physical, Hydrophysical and chemical properties and on the production of some vegetable and field crops by using lysimeters. M.Sc. thesis submitted to the faculty of agriculture. Damascus, University.
4. Hamad Ibtisam, Abdelgawad Gilani, Fares Farouk, 1997. A case study on the use of Barada river water and its use in irrigated agriculture. Damascus University and the Arab Center for Studies of Arid zones and Dry Lands (ACSAD), pp: 120.
5. Prost, R., 1997. Contaminated Soils, proceeding of the 3rd International Conference on the Biogeochemistry of Trace Elements, May 15-19, 1995. INRA-Paris, Versailles 78026, France.
6. Pescod, M.B., 1991. Wastewater treatment and use in agriculture. FAO Irrigation and Drainage, paper No. 47, Rome, pp: 350.
7. FAO., 2000. User manual for irrigation with treated waster water. FAO regional office for the near east. Cairo, Egypt.
8. Papadopoulos, I., 1995. Wastewater management for agricultural production and environmental protection in the Near East Region. FAO Regional office for the Near East. Cairo, Egypt, pp: 50.
9. Hamad Ibtisam, Abdelgawad Gilani and K. Algazeri, 1995. Biological monitoring of vegetable pollution irrigated with waste water and ground water in the proceeding on the use of saline water and treated and untreated waste water. Muscat, Oman, pp: 15-20.

10. Nelson Boyer, W., 2000. Hazards to Wildlife from Soil-Borne Cadmium Reconsidered. J. Environ. Qual., 29: 1380-1384.
11. Catherina, A. and J. Matthew, 2002. Kinetics of Arsenic Adsorption on Goethite in the Presence of Sorbed Silicic Acid. Soil Sci. Soc. Am. J., 66: 818-825.
12. Chang, A., A. Page. and T. Asamo, 1995. Developing human health-related chemical guidelines for reclaimed wastewater and sewage sludge applications in agriculture. World Health Organization, Geneva.
13. Kabata-Pendias, A. and H. Pendias, 1995. Trace Element in Soils and Plant. Revised edition. CRC Press, Boca Raton, FL.
14. Kim, W.I., G.B. Jung and B.Y. Kim, 1999. Heavy Metal Contents of Agricultural Fields in Korea. National Inst. Agr. Sci. Tech., Korea. In: Annual Meeting Abst. ASA-CSSA-SSSA, Salt Lake, Utah. Div. S. 11-P 332. National Academy of Sciences-National Academy of Engineering, 1973.
15. Jnad, I., B. Lesikar, A. Kenimer and G. Sabbagh, 2001. Subsurface drip dispersal of residential effluent: Soil chemical and hydraulic characteristics. J. Am. Soc. Agric. Eng., 44: 1149-1157, 1159-1165.
16. Adriano, D.C., 1986. Trace Elements in the Terrestrial Environment. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, pp: 536.
17. EPA., 1992. Environmental protection agency of USA.
18. Kalra, P.Y., 1998. Reference Methods for Plant Analysis CRC Press, Boca Raton, D.C.
19. Doull, J., C.D. Klaassen and M.O. Amdur, 1980. Casarett and Doull's Toxicology, the basic science of poisons. 2nd Edn., Macmillan Publishing Co, Inc., New York, USA.
20. WHO., 1989. Health guideline for the use of waste water in Agriculture, world health organization, publication. Geneva.
21. Fares, F., A. Elkassi and O. Jouzdan, 1998. Effect of irrigation with untreated, treated water and ground water on vegetable and field crop production quality and their effect on soil chemical and physical properties in lysimeters in the symposium about the treatment of waste water and its use. ACSAD.
22. USDA., 1997. Key to the soil taxonomy, (a.van wambeke) Tech. Monogr. 13, USDA, AID, SMSS, Prog.
23. Alloway, B.J., 1990. Heavy metals in soil. Blackie and London, John Wiley and Sons, Inc. New York, pp: 339.
24. Roberts, A.H.C., D. Longhurst and M.W. Brown, 1994. Cadmium status of soils, plant and grazing animals in New Zealand. New Zealand J. Agric. Res., 37: 119-129.
25. Verma, B.C., 1977. Unimproved turbid metric procedure for the determination of sulfate in plants and soils. Talanta, 24: 49-50.
26. Page, A.L., R.H. Miller and D.R. Keeny, 1982. Methods of soil analysis, Part 2. Chemical and Microbiological Properties 2nd Edition. American society of Agronomy, Inc. Soil Science Society of America, Inc. Madison, Wisconsin. USA.
27. Olsen, S.R. and L.E. Sommers, 1982. Phosphorus. Part 2. Agronomy, No. 9. ASA. Madison, WI. USA.
28. Bremmer, J.M and C.S. Mulvaney, 1982. Nitrogen-total. IN: page (Ed.) methods of soil analysis. Agronomy, ASA, Madison, WI. USA., 9: 595-624.
29. Jackson, M.L., 1959. Soil chemical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.
30. Rhoades, J.D. and M. Polemio, 1977. Determining cation exchange capacity, A new procedure for calcareous and gypsiferous soils. SSSA. J., 41: 524-300.
31. Mulvaney, R.L., 1996. Nitrogen-Inorganic forms. In: Methods of soil analysis. Part3. Chemical Methods (Sparks, D.L. *et al*. Ed.). SSSA Book Ser. 5. Soil Science Society of America, Madison, WI., pp: 1123.
32. Rodier, J., 1978. L'analyse de leau, 6 eme ed., Dunod Techniques, Paris, pp: 146-148.
33. Han, F.X. and A. Banin, 1995. Selective Sequential Dissolution Techniques for Trace Metals in Arid-Zone Soil: The carbonate Dissolution Step. In Common. J. Soil Sci., 26: 553-576.
34. Chapman, H.D. and P.F. Pratt, 1961. Method of analysis for soils, plants and waters. Agr. Pupl., Univ. of Calif., Riverside.
35. Rao, R., P. Thanikaivelan, K. Sreeram and B. Nair, 2002. Green Route for the Utilization of Chrome Shaving (Chromium-Containing Solid Waste) in Tanning Industry. Environ. Sci. Tech., 36: 1372-1376.
36. Sopper, W., E. Seaker and. R.K. Bastian, 1982. Land reclamation and biomass production with municipal wastewater and sludge. The Pennsylvania State University Press, University Park. Pa, 16802, pp: 524.
37. Sopper, W.E., 1994. Manual for the revegetation of mine lands in the Eastern United States municipal biosolids. Pub. National Mine Land Reclamation Center at West Virginia University. WVU National Research Center for Coal and Energy.

38. Hille, D.C., B. Olson and M.G. Rigby, 1981. Accumulation of Cd and Zn in Soil and Vegetation from Long Term Application of Waste Water, Proceedings University of Missouri Annual Conference, USA., pp: 372-282.
39. Abo Rous and Samir Hula, 1996. Heavy levels metals in soil and corn in Egypt. Egypt. Soil Sci., 46: 88-97.
40. Abdelgawad Gilani, 2003. Importance and draw back of treated waste water use on the environment WHO proceedings of the work shop on the use of treated west water in the near east Amman, Jordan, pp: 15-38.
41. Alloway, B.J., 1995. Heavy metals in soil. Chapman and Hall. London, England.
42. Al-Gazaeri, K., 1998. Studying the microbial and heavy metals pollutions in Damascus grown plants irrigated with Barada river polluted water and ground water. MS Thesis. Faculty of Science. Damascus University, pp: 120.
43. Jouret, P., T. Schumacher, A. Boc and. R.N. Reese, 2002. Rhizosphere acidification and cadmium uptake by strawberry clover. J. Environ. Qual., 31: 627-633.

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 62-67, 2007
ISSN 1818-6769

Evaluation of Yellow Sticky Traps on Populations of Some Cotton Pests

Ibrahim Gencsoylu

Department of Plant Protection, Faculty of Agriculture, Adnan Menderes University, Aydin, Turkey

Abstract: Yellow sticky card were placed in horizontal and vertical position at two heights with two positions. The height of yellow sticky traps affected the populations. The largest population was captured at 25 cm above the plants for *Frankliniella* spp. In contrast, *Bemisia tabaci* and *Empoasca* spp. were highest at 30 cm above the ground level. Vertical position in each dimension captured more *Empoasca* and *Frankliniella* spp. than horizontal. However, there were not significant differences on *B. tabaci*. Directions also affected the pests. The largest population of *Empoasca* spp. was captured in north to south direction of dimension 2 whereas more *Frankliniella* spp. population captured in east to west direction. However, more *B tabaci* captured in north to south direction in the dimension 1. This information is useful in the development of sampling techniques to aid the growers in making management decisions against the flying insects in cotton.

Key words: Cotton insects % yellow sticky trap % sampling

INTRODUCTION

Cotton is one of the important industrial plants with the production of 800, 000 ha in Turkey and Aydın province has one of the important places in Aegean Region where it has the second place in the cotton production with 267.000 ha in the country [1]. Insect pests have plagued the cotton growing industry over the years and they are a source of constant concern to growers. Insects such as flower thrip, whitefly and leafhopper cause serious destruction to the cotton plants resulting in a monetary loss to the cotton grower. Those insects are serious economic pests in the cotton fields of Turkey [2, 3] and the growers conduct management measures to control cotton insects.

Sticky traps have been widely used as flight traps to monitor flying insects in many agro-ecosystems and are the preferred more preferable method for some insects in the management systems. They have been used against some pests in ornamental plants [4], celery [5], tomato, potato [6] in both fields and greenhouses. The use of yellow sticky traps to monitor populations of flying pest have been widely used for monitoring whitefly [7], thrips [7, 8] and leafhooper [9].

Yellow color was proven to be the most effective color for attracting flying insects than other colors including yellow-green, orange, green and blue card [10]. Some research has showed that rather than color shape, Height and size of traps were more important for the catching of some adult flying insects. Roa [11] compared sticky traps of different colors for monitoring population of *B. tabaci* on cotton in India. Uthamasamy *et al*. [12] and Nandihalli [13] found that 30 cm above the ground level attracted more adult of *B. tabaci* than traps placed at heights of 60, 90 and 120 cm a ground level on cotton in India. Mensah [9] also mentioned that yellow sticky trap for leafhooper should be placed at heights between 25 and 75 cm above ground level in cotton. For *Frankliniella* spp., Brodsgaard [14] mentioned that trap catches of *Frankliniella occidentalis* (Pergande) were affected by trap height, with the largest catches being obtained in traps placed just above the canopy on cucumber in glasshouse.

In this study I compared the effects of yellow sticky traps at various heights, positions, dimensions and directions for some cotton pests. The findings will be useful in the development of sampling techniques to aid the growers in making management decisions against flying insects in cotton.

MATERIALS AND METHODS

To determine the optimal trap height, position, dimension and direction for placing yellow sticky cards to monitor some cotton pests including flower thrips *Frankliniella occidentalis* Pergande and *F. Intonsa*

Corresponding Author: Dr. Ibrahim Gencsoylu, Department of Plant Protection, Faculty of Agriculture, Adnan Menderes University, Aydin, Turkey

(Thysanoptera: Thripidae), whitefly *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae) and leafhooper populations *Empoasca decipiens* Paoli and *Asymmetrasca decedens* Paoli (Homoptera: Cicadellidae), 10×15 cm and 15×10 cm sticky cards were placed at different heights, positions, dimensions and directions during the 2004 and 2005 cotton-growing seasons at the Agricultural Research Center of Adnan Menderes University, Aydin Province, Turkey. The Nazilli-84S local cotton variety was used and planted on 3 May 2004 and 12 May 2005 with north to south direction.

The study design was randomized complete block with three replicates of each trap height, position, dimension and direction. Trap heights were 30 cm above the ground level and 25 cm above the top of the cotton plant. Some researchers used this heigths for some cotton pests. Yellow sticky card were placed horizontal and vertical position to the ground. Vertical position has two dimensions including 15×10 cm in length and height named as dimension 1 and 10×15 cm in length and height named as dimension 2 whereas horizontal position has dimension 1 (15×10 cm) at both heights. Traps in each dimension have also two directions with east to west and north to south direction. Each sticky trap was placed in each plot (30 rows×25 m long) and 10-m space was left to reduce edge effect. The traps used in the study were constructed from yellow plastic cards with >90% reflectance in visible wavelength spectrum of 540-600 nm and uniformly coated with a thin layer of an adhesive compound mixture of synthetic hydrocarbon polymers (Kapar Organik Tarim Sanayi, Ankara, Turkey) on both side. The traps were suspended on a wooden rod and the wooden frame held the sticky card in all heights, positions dimensions and directions; these could be easily moved up to the stake to keep them 25 cm above the plant canopy at sampling dates. However, traps were fixed on a wooden rod at 30 cm above the ground level. Trap height at 25 cm above the top of plant was adjusted once a week to ensure that the height was maintained at 25 cm above the plant.

Yellow sticky cards were collected each week and the number of flower thrips, whitefly and leafhopper populations per trap per week was determined. Yellow sticky cards were removed and transported back to the laboratory, where they were examined using dissecting microscope. Two species of flower thrips and leafhooper were counted together. Also, some natural enemies captured on the cards. However, they are not counted. The cards changed weekly throughout the entire growing season and finished by the picking of cotton. No insecticides were used throughout the sampling period in both years and all cultural practicies were also done.

The data were subjected to analysis of variance and means were separated by Duncan's multiple range test ($p<0.05$). Data were transformed using $\log(x+1)$ before analysis of variance to correct for heterogeneity of variance. Comparisions of data the trap heights, positions, dimensions and directions were analyzed using SPSS [15] version 9.0.1. Data from each year were analyzed separately.

RESULTS

In 2004 seasonal mean number of *Empoasca* spp., *Frankliniella* spp. and *B. tabaci* on yellow sticky traps are presented in Table 1. As leafhooper *E. decipiens* and *A. decedens* were observed on the yellow sticky traps. Both species counted together and mentioned as *Empoasca* spp. in the Tables. The trap height affected the amount of *Empoasca* spp. on the yellow sticky card. The largest population was captured at 30 cm above the ground level compared to the 25 cm above the cotton plant level. At 30 cm height the largest population was captured in the dimension 2 of vertical position with north to south direction. Positions have also effect on the amount. Total amount of two directions in the dimension 1 and 2 of vertical position captured more *Empoasca* spp. was higher than that of horizontal one. Direction also affected the population in the dimension 1 of each position. East to west captured more population. However, in the dimension 2 of vertical position north to south direction was higher than east to west. At 25 cm above the cotton plant level the largest population was obtained in vertical position compared to the horizontal one (Table 1).

The trap height affected the flower thrips the population. The largest population of flower thrips was observed at 25 cm above the cotton plant level compared to the 30 cm above the ground level. The population was significantly different at 25 cm above the cotton plant level among the position, dimension and direction. Total amount of population in two dimensions of vertical position was higher than in horizontal one. The dimension also affected the amount. The largest population was captured with the dimension 1 and dimension 2 in vertical position. However, the lowest amount was observed in horizontal position. Direction also affected the population at the 25 cm above the plant level. East to west direction captured the largest population in dimension 1 and dimension 2 of vertical position and in horizontal position.

Table 1: Seasonal mean numbers (±SE) of some cotton pests at different heights, dimensions and directions on yellow sticky traps in cotton field, 2004

Heights (cm)	Position	Dimensions (cm)	Directions	*Empoasca* spp.	*Frankliniella* spp.	*Bemisia tabaci*
30 cm above the ground						
	Vertical					
		15×10				
			EW	179.70±18.60ab	71.10±16.80bc	120.20±25.30bc
			NS	170.60±17.70b	29.80±7.40c	155.50±28.40a
		10×15				
			EW	168.50±15.90ab	56.60±14.30c	108.10±20.90c
			NS	228.30±20.70a	28.00±5.90c	120.30±24.60bc
	Horizontal					
		15×10				
			EW	169.30±15.80ab	28.40±6.00c	125.30±23.40bc
			NS	140.20±11.20bc	14.20±4.30c	145.10±26.30ab
25 cm above plants						
	Vertical					
		15×10				
			EW	144.60±14.00bc	172.60±33.30a	21.70±7.10d
			NS	105.40±11.30c	81.60±13.30b	22.70±9.90d
		10×15				
			EW	119.80±12.70c	146.00±25.70a	22.50±8.80d
			NS	145.90±15.30bc	80.40±14.70b	17.90±6.10d
	Horizontal					
		15×10				
			EW	70.10±7.40d	70.30±14.30bc	6.20±0.10d
			NS	75.30±8.20d	10.20±2.40c	8.10±1.20d

[a]Means followed by the same letter are not significantly different ($p<0.05$). EW: east to west, NS: north to south direction

However, there were not significant differences between directions except dimension 1 in vertical position at 30 cm above the ground level (Table 1).

The trap height also affected the population *B. tabaci*. The largest population was captured at 30 cm above the ground level compared to the 25 cm above the cotton plant level. At 30 cm above the ground level the largest population was observed with dimension 1 in north to south of vertical position and and the second one was observed with the same dimension in horizontal position. Total amount of population in two direction of dimension 1 was higher than total amount of dimension 2 in vertical position. However, there were not significant differences between dimension 1 in both positions. Direction affected the population at 30 cm above the ground level and statistical differences were observed. North to south direction in two positions and dimensions were higher than east to west. At 25 cm above the plant level there were not significant differences in positions, dimensions and directions. However, vertical position in two dimensions captured more whitefly populations than the horizontal position (Table 1).

In 2005 seasonal means number of *Empoasca* spp., *Frankliniella* spp. and *B. tabaci* on the yellow sticky traps are presented in Table 2. Trap height affected the amount of *Empoasca* spp. The largest population was captured at 30 cm above the ground level compared to 25 cm above the cotton plant level. At 30 cm the largest amount was obtained in north to south direction of vertical position in the dimension 2. Total amount of vertical position in each dimension captured more population than horizontal one. Direction also affected the amount. The largest population was captured with north to south direction of dimension 2. However, the population in east to west direction in the dimension 1 of vertical and horizontal position were higher than north to south direction. At 25 cm above the plant level the largest population was captured in the dimension 1 of vertical position and statistically different from the other dimensions. There were not statistically differences on the direction except east to west direction of dimension 1 in vertical position (Table 2).

Trap height affected the amount of flower thrips populations captured on the yellow sticky trap. The

Table 2: Seasonal mean numbers (±SE) of some cotton pests at different heights, dimensions and directions on yellow sticky traps in cotton field, 2005

Heights (cm)	Position	Dimensions (cm)	Directions	*Empoasca* spp.	*Frankliniella* spp.	*Bemisia tabaci*
30 cm above the ground						
	Vertical					
		15×10	EW	177.20±19.40ab	22.40±5.20c	161.20±17.00bc
			NS	157.30±17.20b	14.60±3.10c	234.90±26.40a
		10×15	EW	143.10±18.90b	12.90±2.90c	150.60±23.80c
			NS	212.70±25.90a	22.78±6.00c	162.90±16.20bc
	Horizontal					
		15×10				
			EW	164.40±14.50ab	31.70±5.40bc	184.20±30.90bc
			NS	132.10±11.80bc	16.11±3.70c	205.20±31.30ab
25 cm above plants						
	Vertical					
		15×10				
			EW	130.00±14.70bc	167.90±36.50a	10.20±3.20d
			NS	87.70±7.80c	79.20±15.40b	9.20±3.40d
		10×15				
			EW	93.60±9.90c	139.30±30.50a	16.70±4.90d
			NS	93.70±11.50c	77.50±16.20b	12.10±4.30d
	Horizontal					
		15×10				
			EW	79.10±8.50c	81.30±16.30b	5.90±0.90d
			NS	82.50±9.40c	16.10±3.70c	7.30±1.50d

[a]Means followed by the same letter are not significantly different ($p<0.05$). EW: East to west and NS: north to south direction

largest populations were observed at 25 cm above the ground level compared to the 30 cm above the ground. Yellow sticky trap affected the amount of population and statistical differences were observed at 25 cm above the cotton plant. Total amounts of population were captured in vertical than horizontal position in total. Direction in all position and dimension also affected the population and east to west direction captured more flower thrips population compared to the north to south. The largest populations were obtained in dimension 1 with east to west direction and dimension 2 and with east to west direction in vertical position. However, at 30 cm above the ground level there were not significant differences among the positions, direction and dimensions except horizontal's east to west direction (Table 2).

Trap height also affected the amount of *B. tabaci* in 2005. The largest population was captured at 30 cm above the ground level compared to the 25 cm above the cotton plant level. At 30 cm above the ground level there were significant differences on dimensions and directions. In the total amount of vertical and horizontal position there were not significant differences between total amount including both directions of vertical and horizontal position. The dimensions have effect on the population. The largest amount was obtained and in dimension 1 of the 2 position with north to south and they were higher than dimension 2. At 25 cm above the cotton plant level, statistical differences were not observed among the position, dimension and directions. However, vertical position captured more whiteflies on the traps.

DISCUSSION

It was found the vertical position captured more *Empoasca* spp. than the horizontal position in both years. Differences were also obtained in two dimensions of vertical position and the largest populations were obtained in the dimension 1 (15×10 cm). Dimension and height did differ at 25 cm above the plant. Direction of dimension also affected the population level. The largest population was observed in north to south direction of horizontal and vertical position at 30 cm above the ground whereas it was not seen at 25 cm above the plant level. The result showed that the differences were observed mainly on the high population captured on the yellow sticky trap. Atakan and Canhilal [16] reported that leafhooper catch was significantly higher at 60 cm. Mensah [9] said that yellow sticky trap for leafhooper should be placed at heights between 25 and 75 cm above ground level in cotton. The study showed that the largest

population was captured at 30 cm above the ground level and the same result was obtained with some experiments. However, it was concluded that direction affected the population captured no the yellow sticky trap. It was not found any study on the direction that affected the population. Therefore, it was difficult to compare the result of directions and dimensions. I thought that sunlight reflect more light on north to south direction, thus, attracted more leafhooper population than east to west direction.

The height of yellow sticky trap signifcantly affected the amount of seasonal population of *Frankliniella* spp. The largest number of flower thrips population captured at 25 cm above the cotton plant compared to that of 30 cm above the ground level in both years. The research showed that yellow sticky traps affected the capture of flower thrips between vertical and horizontal position and the largest population captured in vertical position and higher than horizontal one. There were significant differences between two dimensions in vertical position. Dimension 1 captured more population. Also, direction affected the population at 25 cm above the cotton plant level and statistical differences were also observed among the dimensions of trap in vertical position and vertical position's east to west direction was about two times more than north to south. However, there were not significant differences among the position's north to south in two dimensions placed vertically at 30 cm above the ground level. The same result also obtained at 30 cm above the ground level. However, it did not differ statistically. Atakan and Canhilal [16] reported that most flights of flower thrips occured above the plant canopy. Pearsall [17] also reported that numbers of flower thrips in flight decreased with height of sticky trap from the ground in nectarine orchards. This study showed that the yellow sticky trap at 25 cm above the plant captured more flower thrips in cotton like the other crops. It was concluded that sticky traps should be placed vertical position with east to west direction for flower thrips in cotton at 25 cm above the cotton plant level.

B. tabaci population were affected from the height, position, dimension and direction of yellow sticky trap in both years at 30 cm above the ground level. The largest population was signifacntly captured at 30 cm above the ground level compared to the 25 cm above the cotton plant in both years. At 30 cm above the ground level there were statistical differences observed between dimensions and the directions of yellow sticky trap at 30 cm above the ground level. It was found that there were not significant differences between total amounts of dimension one of vertical and horizontal position in both years. However, differences were observed in two dimensions of vertical position and the largest populations were obtained in the dimension 1. Dimension and height did differ at 25 cm above the cotton plant. Direction of the dimension affected the population amount. The largest population was obtained with north to south in vertical and horizontal position at 30 cm to ground level. The study showed that the same result was obtained with some experiments. Atakan and Canhilal [16] found that numbers of whiteflies were highest at 60 cm in cotton field. It was concluded that yellow sticky trap should be placed in the dimension 1 of vertical position and with north to south direction for *B. tabaci*.

In conclusion, yellow sticky trap in vertical position placed at 30 cm above the ground level and 25 cm above the plant canopy level will provide a practical method for monitoring the whitefly, flower thrips and leafhooper populations during the growing season. However, the position and height were not enough only. Dimension and direction also play an important role in catching flying insects. This information will be useful in the development of sampling techniques to aid growers in making management decisions against flying insects in cotton.

REFERENCES

1. Anonymous, 2003. Statistical division of Agricultural Department of Aydın province.
2. Atakan, E. and A.F. Özgür, 1999. Determination of oviposition side of *Frankliniella intonsa* (Trybom) (Thysanoptera: Thripidae) J.Turkish Entomol., 23: 135-141.
3. Gencsoylu, I., 2001. A research on population dynamics of cotton pests, relationship with natural enemies and their effects on cotton quality and fibre in different management programs in Buyuk Menderes Valley. Ph.D. Dissertation, Adnan Menderes University Graduate School, pp: 248.
4. Chu, C.C., T.J. Henneberry and E.T. Natwick, 1998. *Bemisia tabaci* adults caught in CC Whitefly traps at different trap heights and trap catch relationships to leaf-turn counts on cotton. Southwestern Entomol., 23: 259-268.
5. Heinz, K.M. and W.E. Chaney, 1995. Sampling for *Liriomyza huidobrensis* (Diptera: Agromyzidae) larvae and damage in celery. Envron. Entomol., 24: 204-211.
6. Zoebisch, T.G., J.L. Stimac and D.J. Schuster, 1993. Methods for estimating adult densities of *Liriomyza trifolii* (Diptera: Agromyzidae) in Staked tomato fields. J. Econ. Entomol., 86: 523-528.

7. Steiner, M.Y. L.J. Spohr, I. Barchia and S. Goodwin, 1999. Rapid estimation of numbers of whiteflies (Hemiptera: Aleuyrodidae) and thrips (Thysanoptera: Thripidae) on sticky traps. Aus. J. Entomol., 38: 367-373.
8. Vernon, R.S. and D.B. Gillespie, 1995. Influence of trap shape, size and background color on capture of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in a cucumber greenhouse. J. Econ. Entomol., 88: 283-288.
9. Mensah, R.K., 1996. Evaluation of coloured sticky traps for monitoring populations of *Austroasca viridigrise* (Paoli) (Hemiptera: Cicadellidae) on cotton farms. Aus. J. Entomol., 35: 349-353.
10. Park, J.D., Y.S. Ku, D.S. Choi and S.S. Kim, 2001. Damaged aspects, seasonal fluctuations, and attractivity of various colors on *Liriomyza trifolii* Burgess (Diptera: Agromyzidae) in Gerbera. Korean J. Appl. Entomol., 40: 97-103.
11. Roa, N.V., A.S. Reddy and K.T. Roa, 1991. Monitoring of cotton whitefly *Bemisia tabaci* Genn. with sticky trap. Madras Agric. J., 78: 1-7.
12. Uthamasamy, S., P. Sivasubramanian and D. Thangaraju, 1990. Monitoring of whitefly (*Bemisia tabaci*) on upland cotton (*Gossypium hirsutum*). Ind. J. Agric. Sci., 60: 744-746.
13. Nandihalli, B.S., B.V. Patil and S. Lingappa, 1993. Population dynamics of cotton whitefly *Bemisia tabaci* (Genn.). Karnataka J. Agric. Sci., 6: 25-29.
14. Brodsgaard, H.F., 1993. Coloured sticky traps for thrips (Thysanoptera: Thripidae) monitoring on glasshouse cucumbers. Bulletin OILB/SROP, 16: 19-22.
15. SPSS., 1999. SYSTAT statistics II. SPSS Inc. Chicago, Ilinois.
16. Atakan, E. and R. Canhilal, 2004. Evaluation of yellow sticky traps at various heights for monitoring cotton insect pests. J. Agric. Urban Entomol., 21: 15-24.
17. Pearsall, I.A., 2002. Daily flight activity of the western flower thrips (Thysa., Thripidae) in nectarine orchards in British Columbia, Canada. J. Applied Entomol., 126: 293-302.

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 68-74, 2007
ISSN 1818-6769

Assessment of Genetic Variation among Jordanian Barely Landraces (*Hordeum vulgare* L.) as Reveled by Molecular Markers

[1]*Aida Al-Nashash,* [2]*Huseain Migdadi,* [3]*Mohamad A. Shatnawi,* [4]*Hani. Saoub and* [5]*Sameer Masoud*

[1,2]National Center for Agricultural Research and Technology Transfer (NCARTT)
[3]Al-Balqa Applied University, Faculty of Agriculture and Technology, Al-Salt 19117, Jordan
[4]University of Muta, [5]University of Jordan NCARTT, Baqa (19381), Jordan

Abstract: DNA genetic variation among eleven selected Jordanian Barley landraces that showed significant morphological differences and the three long-term checks, in addition to two improved varieties (Rum and Acsad-176) was estimated utilizing DNA marker-based Random Amplified Polymorphic DNA (RAPD). Using a set of 5 primers, a total of 349 data points were scored over all of the landraces. The scored data points corresponded to a total of 40 RAPD markers of which 32 markers were polymorphic with a percentage of 80%. A genetic similarity matrix based on Jaccard coefficient was constructed using the generated RAPD data to assess the genetic relatedness. The mean similarity indices ranged from 0.92 to 0.30 with an average of 0.60, which indicated a high DNA polymorphism occurrence among the landraces. Clustering based on genetic similarity indices basically showed clustering of the same row type regardless to collection sites, which indicated that there was agreement between classical classification based on agronomic traits and those generated by RAPD analysis. Of the 16 bulked samples used, there was an average of 96% reproducibility in the two to three replications using five primers. This analysis demonstrated that the RAPD-PCR has proved to be a useful tool to determine the extent of genetic diversity among barely landraces.

Key words: Barley land races % genetic diversity % polymorphism % RAPD-PCR % reproducibility

INTRODUCTION

Evaluation of genetic diversity based on morphological and agronomic characteristics do not usually provide accurate estimates of genetic differences as they are highly influenced by environmental factors. In recent years, DNA based molecular techniques (RFLP, PCR-based markers) have increasingly been used to detect genetic variation either among cultivars or even within closely related individuals [1]. Among these, the Random Amplified Ploymorphic DNA technique (RAPD) gives reasonably reproducible fingerprints of any complex genome.

Traditionally morphological markers, isozymes and protein markers were used for evaluation of genetic diversity [2, 3]. Estimation of genetic diversity based on morphological characters, could be misleading particularly for quantitative traits, which are controlled by multigenes [4] that are highly influenced by environmental factors, developmental stages and management practices [5] Therefore, comparisons should be made between materials measured in different years and different locations, which consuming time and resources [6]. Even if they are used as markers in local races and populations, they may not be appropriate for elite breeding germplasm [7]. Gerdes and Tracy [2] considered morphological markers as poor indicators of genetic distances. Although biological markers, such as isozyme and protein analysis, give better resolution for genetic diversity than morphology [8], they are also subject to developmental and environmental variation [3]. Also they cannot be used to distinguish between closely related accessions because they give low levels of polymorphism and limited number of loci [9]. These limitations in both morphology and traditional biochemical markers have led researchers to adopt other biochemical techniques for reliable identification and evaluation of genetic diversity in germplasm [8]. Among these techniques, the advent of modern molecular marker technology is proved to be a valuable tool in demonstrating genetic diversity at the DNA level [10]. Various techniques have been used to mark individual characters in segregated populations

Correspondence Author: Dr. Mohamad A. Shatnawi, Faculty of Agriculture and Technology, Al-Balqa Applied University, Al-Salt 19117, Jordan

and detect polymorphism at the DNA level [11]. One of the most commonly used PCR techniques are Random Amplified Polymorphic DNA (RAPD) analysis which involves visualization of DNA fragments of different sizes generated by PCR amplification of template DNA using short (usually 10-mer) of primers of arbitrary sequences, that can be separated on agrose gel in the presence of ethidium bromide and visualized under ultraviolet light.

RAPD markers revealed genetic variation and relationship between 19 Hordeum species and subspecies. High levels of variation in fragment patterns were observed both within and among species with most of the primers used and a high reproducibility was observed in all cases [12]. Marillia and Scoles [10] demonstrated that RAPD technology represents a useful and reliable tool for detecting polymorphism for phylogenetic relationship among 39 wild *Hordeum* species, subspecies and cultivated barley. The potential of RAPD markers as tools in barley breeding and pedigree relationships was investigated by Tinker *et al.* [13]. The procedure used in the study was relatively simple and the polymorphism detected was repeatable and stably inherited.

In Jordan, few attempts have yet been made to evaluate and exploit in a systematic way [14], the genetic diversity available in landraces used in the areas that capture maximum diversity of the target crop [15]. The target areas were, therefore, selected to cover the possible range of topography, climate and species concerned. Both Ajlun and Muwaqqar are agro-biodiversity rich in both wild species and primitive forms of cultivated species and both areas are threatened by replacement of improved varieties.

This study aims at determining the extent of genetic variation among the collected Barley landraces at the DNA level utilizing Random Amplified Polymorphic DNA (RAPD) technique.

MATERIALS AND METHODS

Eleven landraces of barley that showed significant morphological differences on field evaluation, three long-term checks (Harmal, Zanbaka and Arta) and two additional improved cultivars (Rum and Acsad-176) were used for RAPD analysis. Of these, nine were six-row type and seven were two-row type. Ten different plants from each landrace and the controls were selected randomly and used in the molecular analysis of variation. Genomic DNA was extracted from 10-day-old etiolated seedling germinated in the growth chamber maintained at 21°C for 10 days. Wizard genomic DNA purification kit was used for DNA isolation according to the instructions provided by the manufacturer (Promega, USA). The DNA concentration and quality was determined with Pharmacia Biotech Gene Quant spectrophotometer and agarose gel electrophoresis.

The standard RAPD protocol recommended by Williams *et al.* [16] was performed with some modifications. RAPD reaction was carried out in reaction volume of 25 µl containing 2.5 µl of 10X already prepared PCR buffer (100 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM $MgCl_2$ and 0.01% (W/V) gelatin), 2.5 µl (2 mM) of deoxynucleotides triphosphate (dNTPs) solution, five picomoles from each arbitrary single 10-base primer (Operon technologies, USA), 0.2 µl of (5 u µl^{-1}) *Taq* DNA polymerase (Promega, USA), one µl (10 ng) of genomic DNA template. Nuclease-free water (Promega, USA) was added to the final volume of 25 µl. Negative control; the reaction mixture without genomic DNA was used for each primer in each PCR reaction to check for DNA contamination. DNA amplification was carried out with MJ-Research Programmable Thermal Cycler (model PTC-100). The PCR program was set as Five minutes at 94°C (initial denaturing step), 42 cycles each one consists of: One minute 94°C (denaturing) One minute 33°C (annealing) Three minute 72°C (extension) and Five minute 72°C (final elongation step). Following amplification, samples were separated by agarose gel electrophoresis. Utilizing 1 kb and 100 bp DNA ladder estimated molecular sizes of the amplification products. The gel was examined and photographed using the gel documentation system (Vilber Lourmat, France).

The presence or absence of DNA bands for each sample was scored by visual inspection of the gel photographs. The data was transformed into a matrix using 1 (present) or 0 (absent) for all tested landraces and fragment sizes. The presence or absence of an amplified fragment was treated as an independent character without consideration of the qualitative aspects of the results i.e. band intensity. Pair-wise comparisons of landraces, based on both unique and shared polymorphic products, were used to generate Jaccard similarity coefficients employing the following formula [17]: JC=N1/(N1+N2+N3), where; N1: number of bands commonly present in individual a and b; N2: numbers of bands present in individual a but not in b and N3: numbers of bands present in individual b but not in a. The similarity coefficients were then used to construct a dendrogram using the SPSS-11.0 PC software for Window computer program.

RESULTS

Quality of extracted DNA has been determined by using 0.7% agarose gel electrophoresis stained by ethidium bromide. The high intensities of DNA bands with minor smears indicate the high molecular weight of extracted genomic DNA with high purity for RAPD analysis.

The purity of extracted DNA was determined spectrophotometically by the ratio of absorbency at 260/280 nm. The ratios at 260/280 nm were mostly 1.8 and this is an acceptable ratio for further analysis [18, 19]. For RAPD analysis, polymorphic marker was defined as an amplified DNA fragment that was present in the bulked DNA sample of at least one individual and was absent in the bulked DNA sample of all individuals. In contrast, markers appearing at random among individuals in all bulk samples will fail to show polymorphism when the bulk samples are compared. Based on this method, RAPD analysis was performed on genomic DNA bulked by Landraces in order to identify RAPD markers that were unique to each of the landrace. Preliminary experiments were conducted to optimize the amounts of template DNA in each PCR reaction using 50, 25 and 10 ng of genomic DNA.

Fifty-eight primers were initially screened using five landraces, which were selected randomly, to determine the suitability of each primer for PCR amplification. Out of 58 primers, five primers (OPA-04, OPA-13, OPF-01, OPN-04 and OPT-13) showed consistently reproducible polymorphic bands and gave repeatable pattern when tested two to three times with the same landraces. These primers were used for further analysis. The fragment sizes of amplified products were estimated using standard curves for bands in the molecular weight marker in each gel. The five primers, which were selected, produced a total of 40 markers, 32 of which are polymorphic using the 16 bulked samples (Table 1). The number of scorable RAPD fragments, generated per primer varied from 4 to 13 with an average of 8 per primer and the number of polymorphic bands per primer ranged from 3 to 12 with an average of 6.4 polymorphic bands per primer. It was found that 20% of the total 40 fragments were shared by all the 16 bulked samples using the five selected primers; therefore, the percentage of polymorphic fragments was 80%.

The size of the scored amplified DNA fragments ranged from 300 to 2000 bp. However, two markers (produced by OPA-04 and OPA-13) had bands with molecular sizes other than the upper limit and were not scored. The matrix of average genetic similarity [17], computed among the studied landraces based on band sharing values is presented in Table 2. The mean similarity indices ranged from 0.30 between samples to 0.92. All samples showed an average of 0.60, which could mean hat the landraces share an average of 60% of their RAPD fragments.

Table 1: Primers used to generate RAPD fragments in barely landraces with data about the total number of amplified bands, polymorphic and monomorphic band identified by primer and size range of scored products

Primer	Total bands	Monomorphic bands	Polymorphic bands	Scored fragments size
OPA-04	13	1.0	12.0	300-2,000 bp
OPA-13	6	3.0	3.0	700-2,000 bp
OPF- 01	7	1.0	6.0	500-2,000 bp
OPN-04	10	2.0	8.0	470-2,000 bp
OPT-13	4	1.0	3.0	900-2,000 bp
Total	40	8.0	32.0	
Mean	80	1.6	6.4	

The relatedness of the different landraces was estimated by a matrix containing digitized scorable bands and analyzed for each accession using the SPSS program. The genetic similarity was calculated using pairwise comparisons of the landraces and the two varieties. The application of this program resulted in the generation of dendrogram clustering of the landraces based on genetic similarity using Jaccard coefficient (Fig. 1).

The results based on the dendrogram showed that the samples were divided into two main clusters at the highest level of hierarchy in the dendrogram (Fig. 1), except (landrace 23) which was separated in its own group. The first cluster consists from all the six row landraces and the two check varieties (Rum and Acsad-176). Within the six-row groups, other qualitative similarities were observed, five sub-clusters, containing nine of six-row barely, with a similarity ranging from 0.92 to 0.72 were formed. This range indicated that landraces of the six-row type are related to each other with high similarity. The second cluster consists of landraces of two-row type only, including the three long-term checks. The similarity between the sub-clusters ranged from 0.82 to 0.52.

In the second cluster, each one of the check-improved landraces made its own cluster, except Arta landrace, which showed high similarity value with (landrace 20). Arta is a single line selection from Syrian barely landrace “Arabi Abiad” [20]. Although a problem in the use of RAPD technique has been DNA pattern

Table 2. Similarity matrix for 11 samples of barely landraces and the checks based on Jaccard Coefficient

Landrace no.	3	4	8	9	11	13	14	20	23	26	32	Harmal	Zanbaka	Arta	Rum
4	0.80														
8	0.89	0.82													
9	0.83	0.76	0.92												
11	0.74	0.73	0.82	0.82											
13	0.66	0.77	0.73	0.73	0.77										
14	0.62	0.61	0.69	0.69	0.73	0.70									
20	0.64	0.69	0.65	0.59	0.63	0.73	0.76								
23	0.39	0.38	0.34	0.30	0.38	0.36	0.42	0.38							
26	0.58	0.52	0.53	0.53	0.56	0.47	0.48	0.45	0.50						
32	0.63	0.61	0.58	0.53	0.56	0.57	0.54	0.62	0.56	0.71					
Harmal	0.58	0.61	0.53	0.48	0.52	0.57	0.43	0.50	0.56	0.60	0.71				
Zanbaka	0.50	0.49	0.50	0.46	0.41	0.35	0.36	0.33	0.47	0.56	0.56	0.61			
Arta	0.62	0.61	0.58	0.53	0.47	0.47	0.44	0.50	0.50	0.65	0.82	0.70	0.71		
Rum	0.67	0.85	0.74	0.68	0.66	0.75	0.58	0.67	0.43	0.48	0.59	0.64	0.50	0.58	
Acsad-176	0.77	0.82	0.67	0.61	0.59	0.61	0.47	0.54	0.48	0.53	0.58	0.69	0.55	0.63	0.74
Mean	0.66	0.66	0.64	0.58	0.57	0.54	0.46	0.50	0.50	0.59	0.65	0.66	0.59	0.60	0.74
Overall mean					0.60										

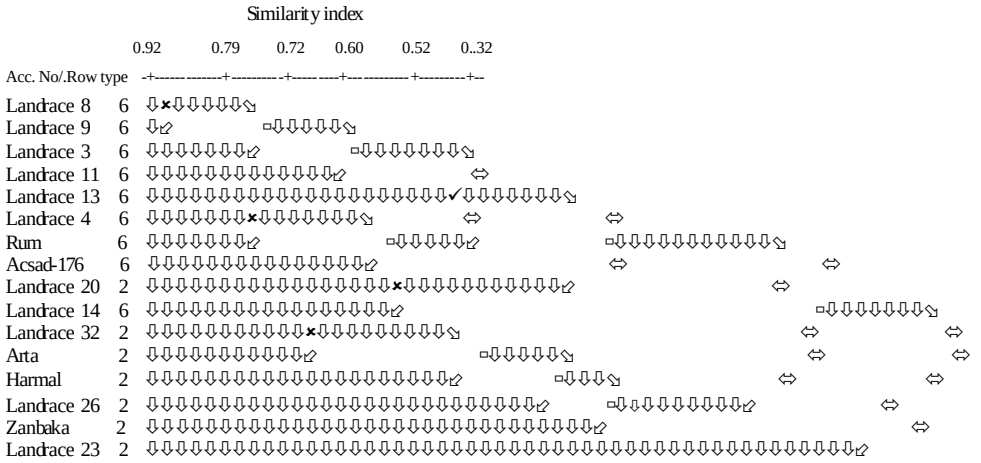

Fig 1: Dendrogram of barely accessions and the controls based on similarity for the 32 RAPD markers produced by five primers

reproducibility, our results demonstrate that RAPD assay can be reproducible with sufficient repetitions (two to three times) in the same laboratory. The 16 bulked samples used showed an average of 96% reproducibility. In the case of reaction failure in one of the three replicates, the scoring was based on the replicate where amplification was successful.

DISCUSSION

The optimum concentration used the lowest amount of template DNA (10 ng) that resulted in the largest number of intense and reproducible bands. In contrast, higher concentrations resulted in non-reproducible or faint bands. This result was in agreement with Weising *et al.* [19] who indicated that for ordinary PCR reaction to be computed, very little amount of DNA was needed. The same results were reported by Abo-elwafa *et al.* [21] and Ko *et al*. [22] who found that the optimum conditions of template DNA concentration were 10 ng/reaction that showed clear banding pattern. However, Hoelzel [18] found that using 100 ng of template DNA per reaction is suitable for RAPD. On the other hand, other concentrations were used for other crop species such as 5-30 ng for pigeonpea [6], one ng for *Aegilops* species [23] and 10-100 ng for wild emmer wheat [24]. In contrast, Schnell *et al*. [24] showed that concentration of template DNA did not affect reproducibility of RAPD markers. The amplified products detected genetic variation among barely landraces in the form of variable number

of different bands [9]. These markers were arbitrarily assumed to amplify only the dominant allele per locus [12, 13]. So, amplification products of any distinct size were assumed to refer to that allele. A locus was considered to be polymorphic if the presence and absence of bands were observed among landraces and monomorphic if the bands were present in all landraces. Since amplified products with large sizes tend to show low reproducibility [9], both faint as well as densely stained RAPD fragments, which were shown in some primers, i.e. OPA-04 and OPA-13, could arise from amplifying two or more products of similar sizes [25]. Variation in the intensity of some bands was also observed in few samples. The possible causes include differences in template sequence copy numbers and varying degrees of mismatch between the primer and the binding sites [6, 26]. The wide range of similarity indices indicated that a high polymorphism at the DNA level among the barely landraces and so, a large amount of genetic variation exists among them.

The genetic similarity was calculated using pairwise comparisons of the landraces and the two varieties. Different authors have used different coefficients to make these estimations: Simple Matching Coefficient [21] Jaccard Similarity Coefficient [9], Nei and Li Coefficient [26], Dice Coefficient [21] or other methods. The most important criterion for the choice of suitable coefficient to apply to RAPD data is that the method does not consider the absence of bands as a similarity. This premise is important in RAPD data analysis because the absence of one band can be due to different mutations which alter the priming site [27]. Therefore, Jaccard Similarity Coefficient, which does not consider absence of bands as a similarity, was used in the present analysis. The clustering was corresponding mainly to the row types of barely. These results agree with that obtained from the cluster analysis of agronomic data (data not shown), which divided the barely landraces into two main clusters according to the row types. Also, the agronomic classification showed that the two-row type harbors more variability than the six-row type.

This study showed that clustering of different landraces did not tend to be clustered according to location of collection, but tend to cluster only according to row-types. This finding is in agreement with our classical classification and that reported by Tinker *et al.* [13]. Whereas Song and Henry [28] demonstrated that the DNA polymorphism of the wild barely correlated with geographical distribution. However, based on RAPD data, Vierling and Nguyen [29] showed that landraces from the same locality tend to cluster together.

Tree topology based on RAPD assay was generally consistent with those based on agronomic treatments, which also divided barely accession into two main groups based on row types. The general good agreement found between classical classification and those produced using RAPDs favors the applicability of the RAPD assay for taxonomic purposes and genetic diversity studies [5, 10, 26, 30]. However, Gonzaler and Ferrer [11] reported RAPD analysis is more accurate in investigating relationships among populations of a single species or very closely related species than between less related species. Although a problem in the use of RAPD technique has been DNA pattern reproducibility, which is greatly influenced by the PCR reaction and amplification conditions in different laboratories [9, 30]. This results demonstrate that RAPD assay can be reproducible with sufficient repetitions (two to three times) in the same laboratory. In the case of reaction failure in one of the three replicates, the scoring was based on the replicate where amplification was successful. Thus under stringent reaction conditions, RAPD pattern could be highly reproducible [27].This demonstrated that RAPD-PCR analysis has proved to be useful in distinguishing among barely landraces that share a high degree of similarity and so, to determine the extent of genetic diversity among them and can provide information for the management of genetic diversity collection and identification [9]. In conclusion, the wide range of similarity indices obtained from RAPD data indicated that high phenotypic and genetic polymorphisms occur among the barely landraces collected in this study, there was an agreement between the classical classification and those produced using RAPD analysis, in which the accession of the six-row type were grouped together in one cluster and clearly separated from accession of the two-row type. The results showed that clustering of different accessions were not based on locations of collection, but mainly according to the row types of and RAPD technique as one of the PCR applications, which should be simple, considered as a useful tool to determine the extent of genetic diversity among barely landraces.

REFERENCES

1. Lowe, A.J.O., O. Hanotte and L. Guarino, 1996. Standardization of molecular genetic techniques for characterization of germplasm collection: the case of random amplified polymorphic DNA (RAPD). Plant Genetic Resources Newsletter., 107: 50-54.

2. Gerdes, J.T. and W.F. Tracy, 1994. Diversity of historically important Sweet corn inbreds as estimated by RFLPs, morphology, isozymes and pedigree. Crop Sci., 34: 26-33.
3. He, G., C.G. Prakash and R.L. Jarret, 1995. Analysis of genetic diversity in a Sweet potato germplasm collection using DNA amplification fingerprinting. Genome, 38: 938-945.
4. Bartolozzi, F., M.L. Warburton, S. Arulsekar and T.M. Gradziel, 1999. Genetic characterization and relatedness among California almond cultivars and breeding lines detected by randomly amplified polymorphic DNA (RAPD) analysis. J. Amer. Soc. Hort. Sci., 123: 381-387.
5. Transue, D.K., D.J. Fairbanks, L.R., Robison and W.R. Anderson, 1994. Species identification by RAPD analysis of grain amaranth genetic resources. Crop Sci., 34: 1385-1389
6. Ratnaparkhe, M.B., V.S. Gupta, M.R. Murthy and P.K. Ranjekar, 1995. Genetic fingerprinting of pigeonpea [*Cajanus cajan* (L.) Millsp.] and its wild relatives using RAPD markers. Theor. Appl. Genet., 91: 893-898.
7. Nybom, H., 1994. DNA fingerprinting - a useful tool in fruit breeding. Euphytica, 77: 59-64.
8. Fuentes, T.L., F. Escobar and M. Cristins, 1999. Analysis of genetic diversity in Cuban rice varieties using isozyme, RAPD and AFLP markers. Euphytica, 109: 109-115.
9. Selbach, A. and S. Cavalli-Molina, 2000. RAPD characterization of Brazilian Barley (*Hordeum vulgare* spp. *vulgare*) Varieties. Euphytica., 111: 127-135.
10 Marillia, E.F. and G.J. Scoles, 1996. The Use of RAPD markers in *Hordeum* phylogeny. Genome, 39: 646-654.
11. Karp, A. and K.J. Edwards, 1995. Molecular techniques in the analysis of the extent and distribution of genetic diversity. In: Ayad, W.G., T. Hodgkin, A. Jaradat and V.R. Rao, (Eds.), Molecular Genetic Techniques for Plant Genetic Resources. Report of an IPGRI workshop, Rome, Italy. International Plant Genetic Resources Institute.
12. Gonzaler, J.M. and E. Ferrer, 1993. Random amplified polymorphic DNA analysis in *Hordeum* Species. Genome, 36: 1029-1031.
13. Tinker, N.A, M.G. Fortin. and D.E. Mather, 1993. Random amplified polymorphic DNA and pedigree relationships in spring barely. Theor. Appl. Genet., 85: 976-984.
14. Malkawi, H.I., M.M. Ajlouni and A.A. Jaradat, 1998. Analysis of genetic diversity of wild emmer and durum wheat by RAPDs. In: Jaradat, A.A., (Ed.), 1998. *Triticeae III*. Science Publishers, Inc. Enfield, NH, USA.
15. Ceccarelli, S., 1984. Utilization of landraces and *Hordeum spontaneum* in barley breeding for dry areas at ICARDA. Rachis, 32: 8-11.
16. Williams, J.G.K., A.R. Kubelik, V.J. Licak, L.A. Ratalski. and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, 18: 6531-6535.
17. Jaccard, A., 1974. The Genetic Structure of Population. Springer Verlag, Berline. German Federal Republic.
18. Hoelzel, A.R., 1992. Molecular Genetic Analysis of Populations. First Edition. Oxford University Press, New York.
19. Weising, K., H.K. Nynom, K. Wolff and W. Meyer, 1995. DNA Fingerprinting in Plants and Fungi. CRC. Press. Inc. USA., pp: 322.
20. Ceccarelli, S., S. Grando and J.A.G. Van Leur, 1995. Barley landraces of the Fertile Crescent offer new breeding options for stress environments. Diversity, 11: 112-113.
21. Abo-elwafa, K, K. Murani and T. Shimada, 1995. Intra- and inter-specific variation in lens revealed by RAPD markers. Theor. Appl. Genet., 90: 335-340.
22. Ko H.L., D.C. Cowan, R.J. Henry, G.G. Graham, A.B. Blakeney and L.G. Lewin, 1994. Random amplified polymorphic DNA analysis of Australian rice (*Oryza sativa* L.) varieties. Euphytica, 80: 179-189.
23. Al-Syouf, M.Q., 2001. Aspects of Ecological Characterization and Introgression from Wheat-Landraces in Triticum Dicoccoides (Korn. ex Asch. & Graebner) Schweinf in Jordan. Ph.D. Dissertation, University of Jordan, Amman, Jordan.
24. Schnell, R.J., C.M. Ronning and R.J. Knight, 1995. Identification of cultivars and validation of genetic relationships in *Mangifera indica* L. using RAPD markers. Theor. Appl. Genet., 90: 269-274.
25. Migdadi, H.M., 2001. Genetic Variation in Some Aegilops Species as Revealed by Morphological and Molecular Techniques. Ph.D. Dissertation, University of Jordan, Amman, Jordan.
26. Kresovich, S., W.F. Lamboy, R. Li, J. Ren, K. Amy, S. McFadden S.M. Bliek, 1994. Application of molecular methods and statistical analysis for discrimination of accessions and clones of vetiver grass. Crop Sci., 34: 805-809.

27. Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. Molecular cloning; a laboratory manual. 2nd Edition. Cold Spring Harbor Laboratory Press, USA.
28. Song, W. and R.H. Henry, 1995. Molecular analysis of the DNA polymorphism of wild barley germplasm using the polymerase chain reaction. Genetic Resources and Crop Evolution., 42: 273-281.
29. Vierling, R.A. and H.T. Nguyen, 1992. Use of RAPD markers to determine the genetic diversity of diploid wheat genotypes. Theor. Appl. Genet., 84: 835-838.
30. Cipriani, G., R.D. Bella and R. Testolin, 1996. Screening RAPD primers molecular taxonomy and cultivar fingerprinting in the genus *Actinidia.* Euphytica, 90: 169-174.
31. Joshi, C.P. and. H.T. Nguyen, 1993. Application of the random amplified polymorphic DNA technique for the detection of polymorphism among wild and cultivated tetraploid Wheats. Genome, 36: 602-609.
32. Okuno, K, K. Ebana, B. Noov and H. Yoshida, 1998. Genetic diversity of Central Asian and north Caucasian *Aegilops* species as revealed by RAPD markers. Genet. Res. & Crop Evolution, 45: 389-394.

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 75-79, 2007
ISSN 1818-6769

The Effect of Tillage Practices on Barley Production under Rainfed Conditions in Jordan

Taha Ahmad Al-Issa and Nezar Husein Samarah

Department of Crop Production, Jordan University of Science and Technology,
Irbid P.O. Box (3030), 22110, Jordan

Abstract: Barley (*Hordeum vulgare* L.) is the most widely grown cereal crop under semi-arid conditions in Jordan. The traditional or conventional tillage systems practiced in Jordan depleted soil resources and resulted in lower crop yields. The use of conservation tillage systems increases the efficiency of soil moisture storage. Therefore, the conservation tillage system is expected to increase crop yield as compared with the traditional tillage systems. A field study was conducted during the growing season of 2002/2004 in Northern Jordan, to investigate the performance of barley under traditional or conventional tillage using a disk plow, conservation tillage using a chisel plow and no-tillage systems in a fallow barley rotation. During the experiment, the soil moisture content for each tillage system was determined at different sampling dates. Number of seedlings m^2, plant height, straw yield and grain yield were determined. The results showed that the conservation tillage system gave the best results concerning soil moisture content, number of seedlings m^2, straw and grain yield compared to the other tillage practices used in the experiment. However, for more sound judgments, the experiment needs to be done for more than one growing season.

Key words: Barley % no-tillage % disk % chisel % conservation % conventional

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the most widely grown cereal crop in Jordan and other West Asian countries. The barley-based farming system exists in wide areas along the dry margins (200-300 mm annual rainfall) of cultivation in Syria, Jordan and Iraq [1]. During the period of 2000 to 2004 the average harvested area of barley in Jordan was 33.13 thousand hectares producing the average amount of 35.4 thousand tons of grain [2]. Lack of soil moisture was identified as the major factor limiting crop growth and production under rainfed conditions [3].

The development of tillage practices for dryland crop production has been and will be a dynamic process. The traditional and exploitive cropping system, which has been practiced in Jordan and other Middle Eastern countries, depleted soil resources and resulted in lower crop yields [4].

Traditional crop rotations practiced in Jordan were based on the fallow system and conventional tillage practices. This system performed reasonably well in the past. However, with the increased population pressure and mechanization of the dryland farming, new highly improved tillage practices are needed to stabilize both soil resources and crop production [5].

The conventional (traditional) tillage systems being used in Jordan consist of land preparation using moldboard plow or disk harrow during October-December to allow growth of weeds after heavy rains followed by hand broadcasting of seeds late November-January and covering seeds by disk harrow. No fertilizers or herbicides are applied in this system [6]. Another tillage system that has been used in Jordan is the conservation tillage or minimum tillage. This tillage system has been defined as reducing tillage only to those operations that are timely and essential to producing the crop and avoiding damage to the soil. Advantages of this tillage system include reduced soil compaction; better soil conservation due to soil roughness and more residue left on the surface and reduced energy requirements. In some instances, yield increases were obtained as a result [5].

No-tillage system refers to a method of planting crops in previously unprepared soil by opening a narrow slot, trench, or band only of sufficient width and depth to obtain proper seed coverage. No other soil

Corresponding Author: Dr. Taha Ahmad Al-Issa, Department of Crop Production, Jordan University of Science and Technology, Irbid P.O. Box (3030), 22110, Jordan

preparation is required and herbicides are used for weed control.

No-tillage soil management has been adopted by many farmers in the US to reduce monetary and external energy inputs, increase profit, conserve soil water and increase soil organic matter [7]. Grain production, which is related to the quantity of crop root and residue inputs to the soil [8], has been shown to increase with no tillage owing to water conservation [9].

Comparisons of zero tillage or direct seeding with conventional or conservation tillage in many studies have indicated that the main benefit of zero tillage is erosion protection through maintenance of surface residue cover. Other benefits include water conservation and reduced labor and fuel costs [10].

In order to determine the tillage practice that is suitable for growing barley in the rainfed areas of Jordan, an experiment was conducted to compare the effect of three different tillage systems on the soil moisture content, yield and yield components of barley grown under rainfed conditions in Jordan. The three tillage systems used are conventional tillage using a disk plow, conservation tillage using a chisel plow and no-tillage systems.

MATERIALS AND METHODS

Site and tillage treatments: The experiment was started in August 2002 at Ramtha Station for Agricultural Research and Technology Transfer, Ramtha (32°30'N, 36°00'E, elevation 590 m), where mean annual precipitation is 250 mm. The soil at the site is a fine, mixed, thermic soil (Typic xerochrepts). The experiment was devised as a Randomized Complete Block Design (RCBD). The experiment consisted of nine plots (three treatments with three replicates per treatment). Each plot was 200 m^2 (10×20 m).

The three tillage treatments were: 1-conservation tillage (CT) using a chisel plow, 2-traditional or conventional tillage (TT) using a disk plow and 3-no-tillage system (NT). The first tillage operation (for tilled plots) was done on August 20, 2002, the second operation on April 12, 2003 and the third one was on August 15, 2003. All plots were planted with "Rum" cultivar using a seed drill at the rate of 100 kg ha^{-1} on November 30, 2003. Germination started on December 14, 2003 in all plots. All plots were fertilized using urea at the rate of 50 kg ha^{-1} on February 18, 2004. For weed eradication, the herbicide 2, 4-D was applied to all plots on January 10, 2004 at the rate of 1 L ha^{-1}.

Data collection: Soil moisture content was measured 17 times during the growing season (October 2003-May 2004). Three soil samples from each plot were taken randomly to the depth of 20-cm for soil moisture content analysis at the following dates: October 14, 2003; December 8, 2003; December 16, 2003; December 21, 2003; December 29, 2003; January 12, 2004; January 18, 2004; January 29, 2004; February 8, 2004; February 17, 2004; February 25, 2004; March 8, 2004; March 30, 2004; April 14, 2004; May 4, 2004; May 12, 2004; and May 30, 2004. Soil moisture content was determined using the gravimetric method.

The numbers of seedlings m^{-2} were taken randomly from each plot on December 18, 2003. The average of three plots count for each tillage system was determined. Plant height in centimeters was measured for five plants randomly selected from each plot on May 12, 2004. Three samples of plants per square meter were harvested on May 17, 2004 from each plot, to measure the yield and yield components of barley. Total biological yield, straw and grain yield were measured in grams m^{-2} for each plot. The average biological yield, straw and grain yield of three plots was determined for each tillage system.

RESULTS AND DISCUSSION

Weather data: Maximum and minimum temperatures and the rainfall during the growing season of the experiment and the average of 24 years for the area are shown in Fig. 1. The maximum temperature was higher than the average for the area in all months of the growing season. The maximum temperature did not differ from the average during the month of December 2003. The minimum temperature was also either more than or equal to the average for the area during the months of the growing season. At the beginning and the end of the season, the rainfall was much below the average for the area. Except for the months of October 2003, January and February 2004, which received the same or little more rainfall than average, the rest of the months (November and December 2003, March, April and May 2004) received lower than the average rainfall of the area for the same period. Figure 1 shows that the amount of rainfall received was not well distributed over the months of the growing season. This means that terminal drought stress occurred during the season.

Soil moisture content: The soil moisture content for the three tillage systems was analyzed using the analysis of variance (ANOVA) method as shown in Fig. 2. The Figure

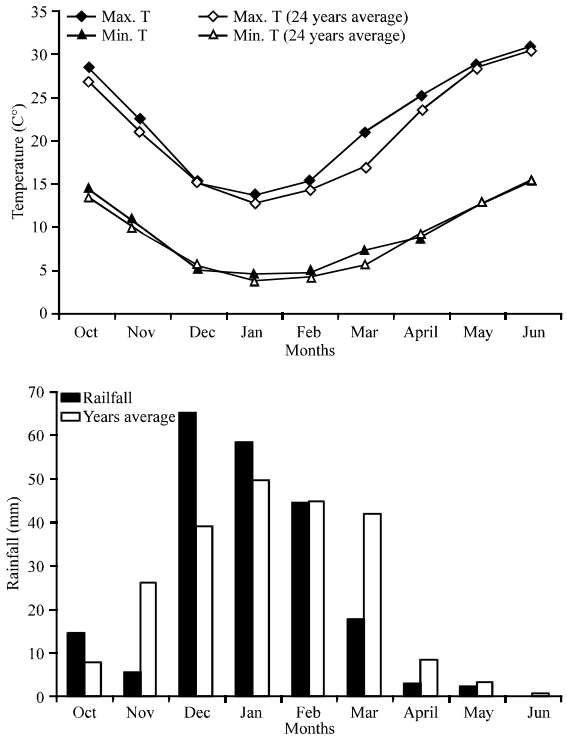

Fig. 1: Maximum and minimum temperatures and rainfall during the growing season of 2003/2004 and the averaged maximum, minimum temperature and rainfall for 24 years

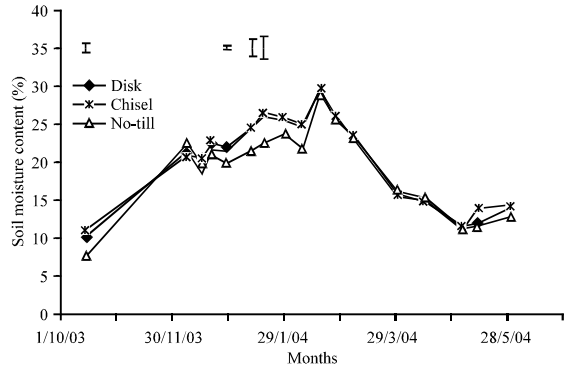

Fig. 2: Soil moisture content at sampling dates for barley fields exposed to three tillage systems in Northern Jordan. Vertical bars indicate the LSD (0.05) when the difference between means was significantly different in ANOVA

shows that the soil moisture content was significantly higher for plots under conservation and conventional tillage systems than non-tilled plots at four sampling dates (October 14, 2003, December 29, 2003, January 12, 2004 and January 18, 2004). There was no significant

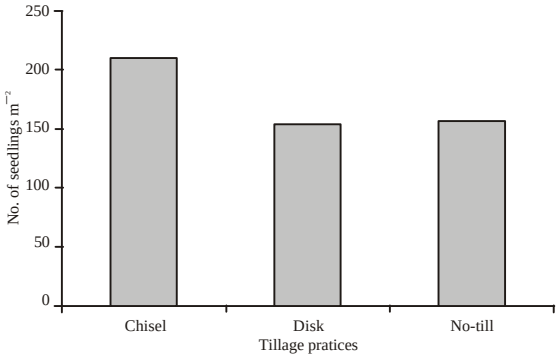

Fig. 3: Number of seedlings for barley grown in fields exposed to three tillage systems in Northern Jordan. Vertical bar indicates the LSD (0.05)

difference in the soil moisture content for the rest of the sampling dates for all tillage systems used in the experiment.

The effect of tillage regime on soil moisture content depended on the time of sampling, soil depth and crop [11]. During the growing season, soil moisture content under conventional tillage (disking) exceeded that under no tillage at the 50-125 mm depth. Rainfall may have percolated in a preferential manner through cracks or root channels beyond this depth under no tillage [12], whereas, soil disturbance with conventional tillage may have resulted in more uniform water movement. In our study, conservation tillage system resulting in higher soil moisture retention, especially early in the season, when rainfall was received most. Soil chiseling appears to promote structure in topsoil, owing to better break-up of large clods and improved resultant water retention characteristics [13].

Seedling establishment: The number of seedlings was measured for all plots under the different tillage systems on January 19, 2004. Figure 3 shows that the number of seedlings per square meter in chisel-plowed plots was significantly higher than those in disked and non-tilled plots.

Some of the concerns with NT, such as delayed emergence, reduced plant population [14] and increased weed and pest populations [15] could be overcome by using a conservational tillage system. In conservation tillage, the improvement in seedling number might be due to improvement in soil structure, seedbed condition and moisture retention.

Plant height: Plant height was significantly higher in plots tilled with a disk plow, followed by plots tilled with

Table 1: Plant height, yield and yield components of barley grown exposed to three tillage practices in Northern Jordan

Tillage systems	Plant height (cm)	Yield components (g m^{-2})		
		Total yield	Grain yield	Straw yield
Chisel	66b	291a	151a	140a
Disk Plow	72a	267a	134ab	133a
No-till	58c	150b	70b	81b

a chisel plow (Table 1). The data in Table 1 supported by previous results, show that conservation and conventional tillage systems improved early establishment and plant growth, resulting in taller plants. Non-tilled plots showed the lowest plant height among all plots.

Soil temperature is an important environmental factor in influencing plant growth. Surface residues in conservation tillage systems decrease soil water evaporation, thus furnishing a more moist and cooler environment [16]. Dao and Nguyen [17] demonstrated that the cool soil temperatures under conservation tillage systems reduced the rate of wheat growth in the early spring. Hay [18] claimed that surface residues lower soil temperature by 2 to 6°C during early spring.

Yield: The total biological yield was significantly higher for plants grown in chisel plowed plots than disked plots and non-tilled plots (Table 1). This is supported by the findings of Al-Issa [19] who found that the use of conservation tillage system produces more wheat yield than the use of conventional tillage system in Northern Jordan. When compared to TT, NT has been found to increase, decrease, or have no effect on the growth and yield of crops [20-24]. The difference in crop responses occur through tillage effects on soil physical, chemical and biological processes and occurrence of crop diseases and may also differ among crops and soils [25-28].

No tillage has been found to diminish soil erosion, but has not always been found to be beneficial for grain yield. Rao and Dao [29] found the no tillage system to occasionally reduce yield through decreased N availability. According to Rasmussen [30], high levels of cereal residues on the soil surface can reduce wheat yield, with reduction variously attributed to disease, weed competition, or decreased light intensity.

CONCLUSIONS

The results of the experiment showed that the conservation tillage system using a chisel plow gave higher soil moisture content, better growth and higher yield of barley than the conventional (disking) and no tillage systems under rainfed conditions. These results indicate that using the conservation tillage system (chisel plow) is the best tillage practice that should be applied to planting barley in the rainfed areas of Jordan. The traditional tillage system using the disk plow and the no-tillage system, were not effective tillage practices for the semiarid region of Jordan.

REFERENCES

1. Jaradat, A.A. and N.I. Haddad, 1994. Analysis and Interpretation of Mashreq Project Findings (1990-92). Special Report: Increased Productivity of barley, pasture and sheep (RAB/89/026). ICARDA-West Asia Regional Program, Amman, Jordan.
2. MOA., 2004. Ministry of Agriculture. Annual Report. Amman. Jordan.
3. Matar, A.E., 1977. Yield and response of cereal crops to phosphorus fertilization under changing rainfall conditions. Agron. J., 69: 879-881.
4. Rafiq, M., 1978. The present situation and potential hazards of small degradation in the countries of the Near East Region. FAO Regional Office for the Near East and UN Environment Program Publications.
5. Jaradat, A., 1988. An assessment of research needs and priorities for rainfed Agriculture in Jordan.
6. Snobar, B.A., 1987. Impact of Mechanization on Wheat Production in Rainfed Areas of Jordan. RACHIS, Vol: 6.
7. Follet, R.F., S.C. Gupta and P.G. Hunt, 1987. Conservation practices: Relation to the management of plant nutrients for crop production. In: Follet, R.F., J.W.B. Sewart and C.V. Cole (Eds.). Soil Fertility and Organic Matter as Critical Components of Production Systems. Soil Sci. Soc. Am. Spec. Publ., Madison, WI, 19: pp: 19-51.
8. Campbell, C.A., F. Selles, R.P. Zentner and B.G. McConkey, 1993. Available water and nitrogen effects on yield components and grain nitrogen of zero-till spring wheat. Agron. J., 85: 114-120.
9. Van Doran, Jr.D.M., Jr.G.B. Triplett and J.E. Henry, 1976. Influence of long term tillage, crop rotation and soil type combinations on corn yield. Soil Sci. Soc. Am. J., 40: 100-105.
10. Larney, F.J., C.W. Lindwall, R.C. Izaurralde and A.P. Moulin, 1994. Tillage systems for soil and water conservation on the Canadian prairie. In: Cater, M.R. (Ed.). Conservation Tillage in Temperate Agroecosystems. CRC Press, Boca Raton, Fl, pp: 305-328.

11. Franzluebbers, A.J., F.M. Hons and D.A. Zuberer, 1995. Tillage and crop effects on seasonal dynamics of soil CO_2 evolution, water content, temperature and bulk density. Applied Soil Ecol., 55: 95-109.
12. Bicki, T.J. and L. Guo, 1991. Tillage and simulated rainfall intensity effect on bromide movement in an argiudoll. Soil Sci. Soc. Am. J., 55: 794-799.
13. McKyes, E., S. Negi, E. Douglas, F. Taylor and V. Raghavan, 1979. The Effect of Machinery Traffic and Tillage Operations on the Physical Properties of a Clay and on Yield of Silage Corn. J. Agic. Engng Res., 24: 143-148.
14. Haynoe, H.N., L.M. Dwyer, D. Balchin, J.L.B. Cully, 1993. Tillage effects on corn emergence rates. Soil Till. Res., 26: 45-53.
15. Blackshaw, R.E., F.O. Larney, C.W. Lindwall and G.C. Kozub, 1994. Crop rotation and tillage effects on weed population in the semi-arid Canadian prairies. Weed Technol., 8: 231-237.
16. Rao, S.C. and T.H. Dao, 1992. Fertilizer Placement and Tillage Effects of Nitrogen Assimilation by Wheat. Agron. J., 84: 1028-1032.
17. Dao, T.H. and H.T. Nguyen, 1989. Growth response of cultivar to conservation tillage in continuous wheat cropping system. Agron. J., 81: 923-929.
18. Hay, R.M.K., J.C. Holmes and E.A. Hunter, 1978. The effects of tillage direct drilling and nitrate fertilizer on soil temperature under winter wheat and barley. J. Soil Sci., 29: 174-183.
19. Al-Issa, T.A., 2001. Farm Machinery Management and the Impact of Conservation Tillage Systems on Soil Erosion and the Sustainability of Wheat Production in Rainfed Areas of Northern Jordan. Ph.D Thesis, Oklahoma State University, Stillwater, Oklahoma, USA.
20. White, P.F., 1990. The influence of alternative tillage systems on the distribution of nutrients and organic carbon in some common western Australian wheat belt soils. Aust. J. Soil Res., 28: 95-116.
21. Carter, M.R., 1991. Evaluation of shallow tillage for spring cereals on a fine sandy loam. 1. Growth and yield components, N accumulation and tillage economics. Soil Till. Res., 21: 23-35.
22. Brandt, S.A., 1992. Zero vs. conventional tillage and their effects on crop yield and soil moisture. Can. J. Plant Sci., 72: 679-688.
23. Carefoot, J.M., H.H. Janzen and C.W. Lindwall, 1994. Crop residue management for irrigated cereals on the semi-arid Canadian prairies. Soil Till. Res., 32: 1-20.
24. Xu, C. and F. Pierce, 1998. Dry bean and soil response to tillage and row spacing. Agron. J., 90: 393-399.
25. Chang, C., C.W. Lindwall, 1979. Effect of long-term minimum tillage practices on some physical properties of a Chernozemic clay loam. J. Soil Sci., 69: 443-449.
26. Karlen, D.L., 1990. Conservation tillage needs. J. Soil Water Conserv., 45: 365-369.
27. McLaughlin, A. and P. Mineau, 1995. The impact of agricultural practices on biodiversity. Agric. Ecosyst. Environ., 55: 201-212.
28. Schoenau, J.J. and C. Campbell, 1996. Impact of crop residues on nutrient availability in conservation tillage systems. Can. J. Plant Sci., 76: 621-626.
29. Rao, S.C. and T.H. Dao, 1996. Nitrogen placement and tillage effect on dry matter and nitrogen accumulation by wheat. Agron. J., 88: 365-371.
30. Rasmussen, P.A., R.W. Rickman and B.L. Klepper, 1997. Residue and fertility effects on yield of no-till wheat. Agron. J., 89: 563-567.

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 80-84, 2007
ISSN 1818-6769

Selenium Uptake by *Allium cepa* Grown in Se-spiked Soils

[1]*Shikha Yadav,* [1]*Saurabh Gupta,* [1]*Ranjana Prakash,*
[2]*Julian Spallholz and* [1]*Nagaraja Tejo Prakash*

[1]Department of Biotechnology and Environmental Sciences,
Thapar University, Thapar Technology Campus, Patiala-147 004, India
[2]Department of Food and Nutrition, College of Human Sciences,
Texas Technical University, Lubbock 79409, USA

Abstract: Selenium (Se) is efficiently transferred through the soil-plant-animal-human system. Geographic differences of Se in soils accounts for most variations in the Se content in foods. Strategies to enhance Se intake include increasing the consumption of high Se foods and food fortification. Se is efficiently taken up by the alliumsand these species produce organo-Se compounds that are active in the chemoprevention and anti-oxidant activity. Here we explore the possibility of growing *Allium cepa* in selenium spiked soils with an objective of introducing this species in seleniferous soils of Punjab. *A. cepa* plantlets showing near uniform height and weight were selected from an agricultural nursery for study. Six plantlets were grown in trays with soils spiked with Se (as selenate) soils of 25 µg gm^{-1} and 50 µg gm^{-1}, with one tray of plantlets maintained as control. Three plantlets were harvested after 30 and 70 days. Total Se was measured in plants and soils using GF-AAS. After 30 days of exposure, the weight of the plantlets exposed to 25 µg gm^{-1} averaged 558 mg with whole plant Se of 278.2 µg gm^{-1}. In plantlets exposed to 50 µg gm^{-1}, The average weight was 547 mg with a whole plant Se of 342.8 µg gm^{-1} with reduced bulb size. Further exposure to 25 µg gm^{-1} for 70 days inhibited growth of the plantlets and increased whole plant Se concentration to 885.4 µg gm^{-1}. Plants exposed to 50 µg gm^{-1} had Se increased to 1280.8 µg gm^{-1}. Conclusions: *Allium cepa* can accumulate Se in proportion to available selenate concentrations in soil. Onions which are commercially viable species can be introduced in the cropping profiles of seleniferous soils of the region for selenium mobilization as well as fortification.

Key words: Nutrient % biofortification % crops % onion

INTRODUCTION

Phytoremediation has received increasing recognition as a low-cost, environment friendly approach for managing the toxic effects of selenium (Se). Plantlets have the ability to absorb and sequester Se and to convert inorganic selenium to volatile forms of organic compounds that are released harmlessly into the atmosphere [1]. Once absorbed by plant roots, Se is translocated to the shoot where it may be harvested and removed from the site [2, 3]. The selenium supply in almost all European countries is below the recommended daily intake. In these countries, selenium fortification of foods and the use of selenium supplements are quite popular to compensate for low Se intake from diets. Wheat (*Triticum aestivum*) is known to be a good source for bioavailable seleniumand many studies have been performed to enrich selenium in wheat by selenium fertilization of the soil [4]. In addition, plantlets such as broccoli (*Brassica oleracea* var. *botrytis L.*), Indian mustard (*Brassica juncea* L.), sugarbeet (*Beta vulgaris* L.), rice (*Oryza sativa* L.), sunflower (*Helianthus annus*), white lupine (*Lupinus albus*) and garlic *(Allium sativum*) are also known to accumulate or fortify selenium in tissues in various forms [5-8]. These studies are of importance as some authors have considered the combination of this enriched material with non-enriched food as a source of selenium supplementation [9, 10].

For most of the world's cultures, alliaceous species are most sought after vegetables for salads and garnishes.

Corresponding Author: Dr. Nagaraja Tejo Prakash, Department of Biotechnology and Environmental Sciences, Thapar University, Thapar Technology Campus, Patiala-147 004, India

It is also one of the major processed vegetable crops being exported from Indian sub-continent to Europe and Americas. Among these species, onion consumption exceeds garlic consumption. Onion also produces greater edible bulb biomass than garlic, making it an additional and perhaps important target for Se-enriched vegetables for human consumption. The chemistry and biochemistry of selenium in these sulfur rich species is more easily expressed in terms of the better-known sulfur chemistry because of the great similarities in chemical properties that selenium and sulfur share by virtue of being adjacent group VIA elements [11-14].

In studies using Se-enriched *Allium* species, demonstrated that organoselenium compounds are more active than S analogs in chemoprevention, suggesting that Se-enriched vegetables may be a better delivery source for organoselenium analogs than the commonly used selenite or selenomethionine [12, 15]. By controlling the intensity and frequency of the crop fertilization with water-soluble selenite salts it is possible to cultivate Se-enriched species with 100-1355 µg gG^{1} of Se [16].

For the past few years the attention of several researchers has been engaged in the study of the role of Se in seleniferous soils and crops of Nawanshahr-Hoshiarpur Region of Punjab, Indiaand its impact on human and livestock health [17-19]. In this area, the average selenium intake of both men and women was more than nine times that in the non-seleniferous areas [20] The malformations appearing in livestock were also attributed to feeding of fodder grown in seleniferous soils [17, 21].

The present study was undertaken to determine the absorption and accumulation patterns of Se in *Allium cepa* (onion) within the harvest period of 30 and 70 days. The study is focused towards a broader objective of introducing this species in Se-impacted areas as selenium fortified vegetables and other crops can result in commercially valuable Se-fortified crop products which can be blended with un-enriched crop produce from non-seleniferous regions.

MATERIALS AND METHODS

Ten day old *Allium cepa* plantlets (variety Nasik Red) were obtained from seed store Patiala. The test soil was taken from agricultural land from non-seleniferous agricultural soils. Trays with capacity of holding 11 kg soil per tray were used for the study for sowing of plantlets. The soils were sieved to remove coarse material and other soils debris before the layout of the plantlets.

Plantlets (6 plantlets per tray) were grown in trays with soil pre-spiked with 25 µg gmG^{1} and 50 µg gmG^{1} of Se as selenate with three trays set for each level of Se. Plantlets were grown in one tray kept as control without Se. The experimental and control plantlets were irrigated at regular intervals with single distilled water. Estimation of selenium in plantlets and soil was carried out before sowing of plantlets and after 30 and 70 days. Plantlets were harvested carefully and soil samples were taken from their respective rhizosphere. The samples were oven-dried (60°C) for 2 days to remove moisture. Dried soil samples were sieved before digestion and dry weight of the whole plantlets were recorded.

1 gm soil sample and whole plant were digested separately in perchloric acid:nitric acid mixture [22]. Digested samples were left for cooling followed by dilution and treatment with 5 ml of 0.2% nitric acid, followed by filtration using 0.45 µm filter. Final dilutions were made by using 0.2% nitric acid. Filtrate was analyzed for selenium content by graphite furnace atomic absorption spectrophotometer (GFAAS-Perkin-Elmer Analyst 600) with detection limit 4 µg lG^{1}. Analytical programming was carried out using WinLab 3.0 software. GF-AAS temperature profiles were-drying at 110°C amd 130°C, pyrolysis at 1300°C, atomization/read step at 1900°C and cleanout at 2400°C. Matrix modifier used was mixture of 5 µg palladium nitrate and 3 µg magnesium nitrate (Sigma-Aldrich). Standard solutions were made from Perkin-Elmer stock AAS standards. Statistical calculations were carried out using GraphPad Prism 4.0.

RESULTS AND DISCUSSION

Observations were taken for the weight of plantlets in triplicates of the control set as well as plantlets harvested after the duration of 30 and 70 days (Fig. 1). Their respective Se concentrations (per gram dry weight) along with the Se levels in rhizosphere soils were estimated using GF-AAS (Fig. 2). Background concentration of selenium in the soil estimated before the start of the experiment was found to be below detection limits.

As indicated in the results, growth was observed of plantlets upto 30 days in 25 µg gmG^{1} which did not further increase by 70 days. However, growth at 50 µg gmG^{1} showed retardation. In case of control set, plants had considerable bulb formation with normal leaves and root density. Reduction in bulb size, length of leaves and root density was noted in plantlets grown in soil spiked with 50 µg gmG^{1} Se. Similar visible reduction in foliar mass

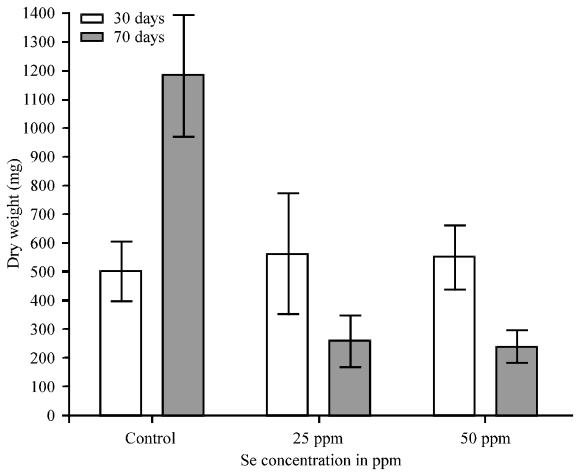

Fig. 1: Plant dry weight (mg) in control and selenium exposed plantlets

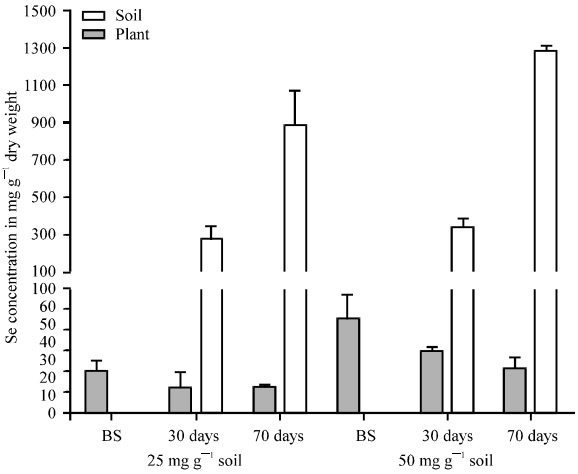

Fig. 2: Selenium levels in soil and plants before sowing (BS) and after 30 and 70 as of growth

was observed under high Se treatments (up to 20 ppm) in "Granex" onions by Kopsell and Randle [14] The researchers suggested that the reduction in biomass might be due to interruption of sulphur metabolism through replacement of S proteins by Se amino acids, producing retarded growth effects. Sulphur metabolism in onion supports plant growth and fertility levels [23, 24]. Decrease in plant growth and yield with increased Se application were also reported for alfalfa (*Medicago sativa* L. Var. African) and subterranean clover (*Trifolium subterraneum* L. var. Mt. Baker) [25].

The estimation of the accumulation of Se content in onion plantlets showed that onions take up and accumulate Se in proportion to available selenium (Na_2SeO_4). Uptake also increased with age of plant. Observations showed that average accumulation of Se in plantlets after 30 days in 25 µg gm^{-1} was 278.7 µg and in 50 µg gm^{-1} plantlets was 343 µg After 70 days, it was 885.5 µg and 1280.8 µg respectively. Two-way ANOVA analysis of the triplicate samples taken at 30 and 70 days indicated that there is interaction ($p>0.05$) between selenium concentration and duration of exposure on the Se levels in *Allium* biomass. Similar observations in increasing uptake of Se with increase in age of plant were recorded by Kopsell and Randle [14]. Whanger *et al*. [26] also reported that the Se uptake in ramps (*Allium tricoccum*) increased despite difference in the growth media, namely peatmoss (I), vermiculite (II) and hydroponics (III). In I and II, Se added to mixture was 30, 60, 90, 120 mg kg^{-1} and content of Se in ramp bulb was 120, 140, 177, 235 mg kg^{-1} respectively. In case III, concentration of Se added to nutrient solution was 10, 20, 30, 50, 70, 90 mg L^{-1} and content of Se in bulbs was observed to be 88, 142, 252, 325, 335, 432 mg kg^{-1}. The results of the present study also indicated that as growth in the biomass of *A. cepa* does not get affected upto 25 µg gm^{-1} within harvesting time of 30 days. Therefore these species can be grown in seleniferous soils or Se spiked soils and harvested at green onion stage, with medium bulb size for further blending and processing.

Crop plants such as alliaceous species with an augmented capacity to accumulate Se from soils can thus be used to aid sustainable agriculture and to improve human health through balanced mineral nutrition vis-à-vis to mobilize selenium from seleniferous soils, thus using the phytoremediation and biofortification concepts together. *A. cepa*, examined in the present study exhibits potential to mobilize selenium from soil as well as accumulate in the biomass. If the concepts of fortification by plants which have augmented capacity to accumulate Se hold a market value in terms of crops that can be used as Se supplements, these food products can be exported in the specific geographical regions which are naturally deficient for Se. This will serve two main purposes-(a) remediation of selenium-laden soil thereby putting an end to the trauma faced by human and livestock of the region affected by selenium impactand (b) raising financial standards of the farmers of affected regions if such land is taken up for research, consequently for projects dealing with production of export quality fortified foods used as supplements to compensate for low Se intake from diets.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support by CSIR, Govt. of India through sponsored project. The analytical facilities provided by CORE-Agro-Industrial Biotechnology, Patiala for the study are also duly acknowledged.

REFERENCES

1. Bañuelos, G.S., 2002. Irrigation of broccoli and canola with boron and selenium-laden effluent. J. Environ. Qual., 31: 1802-1808.
2. Bañuelos, G.S., 2001. The green technology of selenium phytoremediation. Bio-Factors, 14: 255-260.
3. Wu, L., 2003, Review of 15 years of research on ecotoxicology and remediation of land contaminated by agricultural drainage sediment rich in selenium. Ecotoxicol. Environ. Safety, 57: 257-259.
4. Lintschinger, J., N. Fuchs, J. Moser, D. Kuehnelt and K.F. Moosham, 2000. Selenium-enriched sprouts-A raw material for fortified cereal-based diets. J. Agric. Food Chem., 48: 5362-5368.
5. Zayed, A., C.M. Lytle nd N. Terry, 1998. Accumulation and volatilization of different chemical species of selenium by plants. Planta, 206: 284-292.
6. Kotrebai, M., M. Birringer, J.F. Tyson, E. Block and P.C. Uden, 2000. Selenium speciation in enriched and natural samples by HPLC-ICP-MS and HPLC-ESI-MS with perfluorinated carboxylic acid ion-pairing agents. Analyst, 125: 71-78.
7. McSheehy, S., W.J. Yang, F. Pannier, J. Szpunar, R. Lobinski, J. Auger and M. Potin-Gautier, 2000. Speciation analysis of selenium in garlic by two-dimensional high-performance liquid chromatography with parallel inductively coupled plasma mass spectrometric and electrospray tandem mass spectrometric detection. Analytica Chimica Acta, 421: 147-153.
8. Roberge, M.T., A.J. Borgerdingand J.W. Finley, 2003. Speciation of selenium compounds from high selenium broccoli is affected by the extracting solution. J. Agric. Food Chem., 51: 4191-4197.
9. Guerinot, M.L. and D.E. Salt, 2001. Fortified foods and phytoremediation: Two sides of the same coin. Plant Physiol., 125: 164-167.
10. Ximeanez-Embuan, P., I. Alonso, Y. Madrid-Albarraän and C. Caä Mara, 2004. Establishment of selenium uptake and species distribution in lupine, indian mustardand sunflower plants. J. Agric. Food Chem., 52: 832-838.
11. Lauchli, A., 1993, Selenium in plants: Uptake, functionsand environmental toxicity. Botanica Acta, 106: 455-468.
12. Ip, C. and D.J. Lisk, 1994a. Characterization of tissue selenium profiles and anticarcinogenic responses in rats fed natural sources of selenium-rich products. Carcinogenesis, 15: 573-576.
13. Gutenmann, W.H. and D.J. Lisk, 1996. Increasing selenium in field grown onions by planting in peat moss pots containing coal fly ash. Chemosphere, 32: 1851-1853.
14. Kopsell, A. and J. Randle, 1997. Selenate concentration affects selenium and sulfur uptake and accumulation by 'Granex 33' Onions. J. Am. Soc. Hortic., 122: 721-726.
15. Ip, C. and D.J. Lisk, 1994b. Enrichment of selenium in allium vegetables for cancer prevention. Carcinogenesis, 15: 1881-1885.
16. Ip, C. and D.J. Lisk, 1995. Efficacy of cancer prevention by high selenium garlic is primarily dependent on the action of selenium. Carcinogenesis, 16: 2649-2652.
17. Dhillon, K.S. and S.K. Dhillon, 1991. Selenium toxicity in soil, plants and animals in some parts of Punjab. Intl. J. Environ. Studies, 37: 15-24.
18. Srivastava, A., A. Kumar, Y.M. Scindia, A.G.C. Nair and A.V.R. Reddy, 2002. Multielement analysis of soils from North western region of India using NAA with special reference to selenium toxicity. J. Radioanalytical and Nuclear Chem., 254: 645-648
19. Srivastava, A., N. Tejo Prakash, S. Bhatnagar, A. Kumar, S. Gupta, U.S. Sadana and J.E. Spallholz, 2006. Selenium toxicity in water, soils and crops in the Nawanshahr region of India, In: Proceedings of International Symposium on Selenium in Biology and Medicine-Selenium 2006, USA, pp: 120.
20. Hira, C.K., K. Partal and K.S. Dhillon, 2003. Dietary selenium intake by men and women in high and low selenium areas of Punjab. Public Health, 7: 39-43.
21. Dhillon, K.S., N.S. Randhawa and M.K. Sinha, 1977. Selenium status of some common fodders and natural grasses of Punjab. Ind. J. Dairy Sci., 30: 218-224.
22. USEPA, 1996. Acid digestion of sediments, sludges and soils. Method 3050-B. In: Methods for chemical analysis of water and wastes. USEPA, Washington, DC.
23. Landcaster, J.E. and M.J. Boland, 1990. Flavor biochemistry, In: Onions and allied crops. Rabinowitch, H.D., J.L. Brewster (Eds.). Boca Raton, USA: CRC Press, pp: 33-72.

24. Randle, W.M., E.B. Block, M.H. Littlejohn, D. Putman and M.L. Bussard, 1994. Onion *(Allium cepa* L.) thiosulfinates respond to increasing sulfur fertility. J. Agric. Food Chem., 42: 2085-2088.
25. Broyer, T.C., D.C. Lee and C.J. Asher, 1966. Selenium nutrition of green plants. Effects of selenite supply on growth and selenium content of alfalfa and subterranean clover. Plant Physiol., 41: 1425-1428.
26. Whanger, P.D., C. Ip, C.E. Polan, P.C. Uden and G. Welbaum, 2000. Tumorigenesis, metabolism, speciation, bioavailabilityand tissue deposition of selenium in selenium-enriched ramps (*Allium tricoccum*). J. Agric. Food Chem., 4: 5723-5730.

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 85-89, 2007
ISSN 1818-6769

$-Glucan Sex-Dependently Attenuates the Hyperalgesic Effects of Arsenite in Rats

[1]A.S. Oyadeyi, [2]V.A. Togun, [3]J.K. Oloke and [3]O.G. Oyeyiola

[1]Department of Physiology, Faculty of Basic Medical Sciences,
[2]Department of Animal Production and Health, Faculty of Agricultuiral Sciences,
[3]Biotechnology Center, Department of Pure and Applied Biology,
Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso. Oyo State, Nigeria

Abstract: Against a backdrop of arsenite neurotoxicity, we investstigated the effects of arsenite on pain processing and whether the effects could be reversed by the administration of $-glucan, an antioxidant from Saccharomyces Cerevisae. Arsenic (5 mg kg^{-1}) significantly reduced tail flick latency 48 h after administration in animals. The hyperalgesia appeared sex-specific as female rats showed higher degree of hyperalgesia than male rats. Direct oral administration of $-glucan (0.5 mg kg^{-1}) significantly increased tail-flick latency while indirect administration through feeding of Saccharomyces Cerevisae-digested feed failed to produce any significant attenuation of the hyperalgesia. The analgesia produced by $-glucan also appeared to be sex specific. We conclude that arsenic produces hyperalgesia in rats probably through oxidative damage and that the hyperalgesic effect are reversible by antioxidants.

Key words: Arsenite % $-glucan % pain % hyperalgesia % nociception

INTRODUCTION

Humans and animals live in a complex environment and are subjected to various biological, Physical and environmental stimuli. Exposure to a variety of laboratory and environmental factors such as arsenite has been shown to induce a number of behavioural and physiological responses including alterations in nociceptive processing [1-3]. Arsenic is a nonessential trace element; it is a potent, toxic, mutagenic and xenobiotic metalloid. Arsenite has been growing rapidly during the last 5 years as a major pollutant of drinking water in several regions of the world [4, 5].

Arsenic, as trivalent arsenite (As^{3+}) or pentavalent arsenite (As^{5+}) is naturally occurring and ubiquitously present in the environment. Humans are exposed to arsenic mainly through either oral or inhalation routes. Oral exposure occurs via consumption of contaminated water, food and drugs [6]. Arsenic has been claimed to be of clinical utility in the treatment of syphilis, amoebiasis and certain other tropical diseases [7] and has also been used in Fowler solution in the treatment of arthritis [7]. Arsenic exposure results in endemic arsenic dermatosis along with hyperkeratosis, gangrene and skin cancer [8]. Arsenic intoxication in experimental animals has recently been associated with hepatic tumors [9], the inhibition of testicular steroidogenic function [10], incapacitation of Leydig cell function, negative effects on caudal epididymal milieu [11] and spermatogenesis [12]. It has also been implicated with severe metabolic disorders such as diabetes in humans [13]. Arsenic exposure has been reported to result in structural changes in the thymus of pregnant and newborn mice [14]. Long-term exposure of arsenic is associated with abortion, low birth weight and reduced lactation [15] as well as with embryonic cells toxicity *in vitro* [16]. Survey reports from the Ukraine, Taiwan and Bangladesh revealed that the intake of arsenic-contaminated drinking water caused reproductive disturbances in women [17], adverse pregnancy outcomes [18] and spontaneous abortion [19]. Acute arsenic exposure may promote immediate gastrointestinal tract infection [20], while chronic effects may exert degenerative, inflammatory and neoplastic changes of the respiratory, haematopoetic, cardivascular and nervous systems [21]. There is however a lack of literature data on the possible effects of arsenate on nociceptive processing, a possibility that is very strong in the light of arsenite-induced inflammatory perturbations on one hand [21] and its peripheral neuropathic effects on the other [6]. Peripheral neuropathy, among other

Correspondence Author: Dr. A.S. Oyadeyi, Department of Physiology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Oyo State, Nigeria

impacts on the sensory system, will most definitely affect nociceptive processing because of the close association between the state of peripheral nerves and the sensory modality they transmit.

The deleterious effects of arsenite have been attributed to oxidative damage, thus an antioxidant should protect against or reverse its toxicity. The need for preventing arsenite-induced toxicity is underscored by the fact that there are no animal models to study the mechanisms of its toxicity while there is an increase in the population of those who are at risk of arsenite poisoning, especially in the developing world [22].

In an on-going effort to understand the mechanisms of arsenite poisoning as well as preventing it, we examined the effects of arsenite on nociception and the possibility of using $-glucan, a potent anti-oxidant, to prevent its toxic effects on nociception. As a secondary aim, we also attempted to determine whether the method of obtaining $-glucan had any effect on its potency.

MATERIALS AND METHODS

Animals: Adult male and female Sprague-Dawley rats were used. They were kept in a temperature (20-21°C) and light-controlled (12 h light:12 h dark cycle) room with free access to food and water.

Synthesis of $-glucan: $-glucan was synthesized as previously described by Hunter *et al.* [23]. Briefly, active dry yeast was added to 0.1 mol G^{1} of NaOH and stirred for 30 min at 60°C. The material was then heated to 115°C at 8.5 psi for 45 min and then allowed to settle for 72 h. The sediment was re-suspended and washed in distilled water by centrifugation (350 g for 20 min). The alkali insoluble solids were with mixed 0.1 mol lG^{1} acetic acid and heated to 85°C for 1h, then allowed to settle at 38°C. The acid insoluble solids were drawn off and centrifuged as above. The compacted solid material was mixed with 3% H_2O_2 and refrigerated for 3 h with periodic mixing. The material was then centrifuged and the pellet washed twice with distilled water, followed by two washings in 100% acetone. The harvested solid material was dispersed on drying trays and dried under vacuum at 38°C for 2 h in the presence of Ca_2SO_4. It was then further dried overnight under vacuum at room temperature.

Synthesis of $-glucan indirectly from Saccharomyces cerevisae (SC)-digested feed: $-glucan was also synthesized indirectly by using SC to digest rice bran as previously described by Iyayi and Aderolu [24]. Briefly the rice bran was dried to constant weight at 60°C; 25 kg of the rice bran was autoclaved and oven-dried. The autoclaved material was then innoculated with SC under aseptic conditions after adjusting the moisture level to 25%. After 14 days, the biodegradation reaction was stopped and the material was then dried.

Nociceptive test: Nociceptive test was carried out by a modification of D'Amour and Smith's tail flick test [25]. Each animal was gently hand-held in a dry towel while the distal two-third of the tail was immersed in water maintained at 50±1°C. The time it took the animal to flick out its tail from the water was recorded as tail-flick latency.

Protocol: The animals were randomly divided into 4 groups and after basal nociceptive threshold was taken for all the rats, those in group I (n=8) were administered 0.2 ml of normal saline. Group II (n=7) were fed digested rice bran in addition to normal rat chow. Group III (n=8) served as control while Group IV rats (n=7) were administered 0.5 mg kgG^{1} (p.o) of the synthesized $-glucan.

Immediately after, all the groups received 5 mg kgG^{1} (i.p) arsenite. They were returned to their home cages and nociceptive testing was carried out 48 h later using the tail-flick test.

RESULTS

Effects of $-glucan on arsenite-induced hyperalgesia: Table 1 shows the baseline tail-flick latency in all animals.

Arsenic significantly produced hyperalgesia 48 h after administration in control animals (Table 2) of both sexes. This level of hyperalgesic effects appeared to be related as female animals showed higher degree of hyperalgesia than males.

Direct $-glucan significantly reduced the hyperalgesia while indirect administration via digested

Table 1: Baseline Tail flick latency in all groups of animals

	Tail flick latency (Secs)	
Groups	Male	Female
CNS	27.94±1.02	24.14±0.41
PRB	39.80±0.58	39.00±2.01
PBE	24.62±1.52	11.02±1.17
CSA	31.60±1.86	24.24±0.63

CNS - Arsenite + normal saline PRB - Arsenite + digested feed

PBE - Arsenite + $-glucan CSA - Arsenite alone

Table 2: Tail flick latency after 48 h

	Tail flick latency (Secs)	
Groups	Male	Female
CNS	28.10±1.01	24.20±0.52
PRB	17.44±0.25*	34.40±1.32*
PBE	32.78±0.33*	13.52±1.14*
CSA	22.16±0.69	12.58±1.20*

*Significant, compared with control, p< 0.01, student t-test

CNS-Arsenite + normal saline PRB-Arsenite + digested feed

PBE-Arsenite + $-glucan CSA-Arsenite alone

Table 3: Percentage effects on nociceptive processing in males and females

	Males	Females
CNS	0.50% Analgesia	0.24% Analgesia
PRB	56.18% Hyperalgesia	11.79% Hyperalgesia
PBE	33.14% Analgesia	22.69% Analgesia
CSA	29.88% Analgesia	75.66% Analgesia

feed failed to produce any significant alteration on hyperalgesia (Table 3). This analgesia effect was more prominent in the male animals than the females.

DISCUSSION

Arsenic produced a conspicuous hyperalgesia in rats. This hyperalgesia was evident in the significantly reduced tail-flick latency. Arsenic is however not the only substance that can cause facilitated nociceptive processing. Acute intra-peritoneal vitamin C [26] and acute restraint stress [27] have also been reported to induce hyperalgesia, although the underlying mechanisms may be different.

Hyperalgesia, generally occurs when the firing threshold of A* and C nociceptive afferent is lowered into the non-noxious range [28]. The mechanisms involve synthesis of arachidonic acid from membrane lipids via the steroid-sensitive enzyme Phospholipase A_2. Arachidonic acid is acted upon by the cyclooxygenase enzyme to produce prostaglandins, which act directly on the peripheral terminals of A* and C fibers and then lower their threshold [28]. Although we are not aware of any report that has examined the effect of arsenite on nociception, several links are possible. For instance, it has been severally documented that arsenite is a potent cytotoxic agent, whose cytotoxicity is not only rapid, as fast as 5 minutes after treatment [6] but also involves reactive oxygen species [22] which induce oxidative damage. Oxidative damage has been implicated in neurological disorders such as arthritis [29]. It is possible that arsenite induces hyperalgesia by damaging peripheral nerves.

Sex differences in nociception have been documented by several investigators with females more generally responsive to pain [30]. The result of higher arsenite-induced hyperalgesia in females compared to males, is consistent with the gamut of evidences that has shown females to respond more to noxious stimuli than males. Although we did not examine the mechanisms, gonadal hormones [31], menstrual cycle [32] and psychosocial factors [33] are some of the factors documented as been responsible for gender differences in pain processing. Our results also show greater analgesic effects of direct $-glucan in males than females. If we take $-glucan as a pharmacologic agent and it is, then this is consistent with studies that have also documented differential analgesic responses in males and females. For example, Icero *et al.* [34] have reported enhanced sensitivity to morphine in males compared to females, a fact that has been subsequently confirmed [35].

The lack of significant effect on account of the digested rice bran consumed by the rats is not surprising. In all reality, the time lapse of forty eight hours was too little for any significant deposition of $-glucan or any other fungal metabolite for that matter. One study that reported enhanced feeding value of rice bran after fermentation with Trichoderma viridae was carried out over several days [24]. It is known that the ability of fungi to degrade fiber lasts several days [36, 37]. Even if there had been adequate digestion of rice bran by Trichoderma viride, the short period of feeding would not have guaranteed sufficient intake of the substance contained in the feed by the animals.

In summary, we report the ability of $-glucan to sex dependently attenuate the hyperalgesia induced by arsenite in rats. This is in concord with reports that have shown that many fungal metabolites can be utilized to treat a wide variety of diseases like inflammation and arthritis [38].

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of the following students: Owojaiye Kehinde, Olarotimi Tolulope, Adurodolorun Olubukola, Eniolorunda Olayinka, Olawumi Oluwaseun, Aroyehun Adeola, Ajonijebu Chris.

REFERENCES

1. Amit, Z. and Z.H. Galina, 1986. Stress-induced analgesia: adaptive pain suppression. Physiol. Rev., 66: 1091-1120.
2. Bodnar, R.J., 1986. Neuropharmacological and neuroendocrine substrates of stress-induced analgesia Ann. N.Y. Acad. Sci., 467: 345-360.
3. Lewis, J.W., 1986. Multiple neurochemical and hormonal mechanisms of stress-induced analgesia. Ann. N.Y. Acad. Sci., 467: 194-204.
4. Chappell, W.R., B.D. Beck, K.G. Brown, R. Chaney, C.C. Richard, K.J. Irgolic and D.W. North, 1997. Inorganic arsenic: A need and an opportunity to improve risk assessment. Environ. Health Perspect., 105: 1060-1065.
5. Nickson, R., J. McArthur, W. Burges, K.M. Ahamed, P. Ravenserof and M. Rahman, 1998. Arsenic poisoning of Bangladesh ground water. Nature, 395: 338-338.
6. Liu, S.X., M. Athar, I. Lippai, C. Waldren and T.K. Hei, 2001. Induction of oxyradicals by arsenic: Implication for mechanism of genotoxicity. Proc. Natl. Acad. Sci., 98: 1643-1648.
7. Klaassen, C.D., 1990. Heavy metals and heavy metal antagonists. In The Pharmaceutical Basis of Therapeutics (G.A. Goodman, T.W. Rall, A.S. Nies and P. Taylor, Eds.). Pergamen Press, New York, pp: 1602-1605.
8. Chowdhury, T.R., B.K. Mandal, G. Samanta, G.K. Basv, P.P. Chowdhury, C.R. Chanda, N.K. Karan, D. Lodh, R.K. Dhar and D. Das, 1997. Arsenic in ground water in six districts of West Bengal, India - The biggest arsenic calamity in the world: The status report up to August, 1995. In Arsenic Exposure and Health Effects (C.O. Abernathy, R.L. Calderson and W.R. Chappell, Eds.). Chapman and Hall, New York, pp: 93-111.
9. Waalkes, M.P., J.M. Ward, J. Liu and B.A. Diwan, 2003. Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary and adrenal tumors in mice. Toxicol. Appl. Pharmacol., 186: 7-17.
10. Sarkar, M., N.M. Biswas and D. Ghosh, 1991. Effect of sodium arsenite on testicular $^{5\text{G}}$3ß, 17ß-HSD activities in albino rats: Dose and duration dependent responses. Med. Sci. Res., 19: 789-790.
11. Togun, V.A., A.S Oyadeyi, J.K Oloke, A.O. Oyewopo, 2005. Effect of sodium arsenite with or without vitamin E on epididymal sperm motility and concentration in Sprague-Dawley rat. Proceedings of the 39th Conference of the Agricultural Society of Nigeria, pp: 57-58.
12. Sukla, J. P. and K. Pandey, 1984. Impaired spermatogenesis in arsenic-treated fresh water fish *Colisa fasciatus (*Bl and Sch). Toxicol. Lett., 21: 191-195.
13. Tseng, C.H., C.P. Tseng, H.Y. Chiou, Y.M. Hsueh, C.K. Chong and C.J. Chen, 2002. Epidemiologic evidence of diabetogenic effect of arsenic. Toxicol. Lett., 133: 69-76.
14. Skal'naia, M.G., A.A. Zhavoronkov and A.V. Skal'nyi, 1995. Morphologic characteristics of the thymus in pregnant and new born mice. Arkhiv Patologii, 57: 52-58.
15. Donald, M.C., R.A. Edwards and J.F.D. Greenhalgh, 1995. Minerals. In Animal Nutrition (M.C. Donald, R.A. Edwards and J.F.D. Greenhalgh, Eds.). Longman ELBS, England, pp: 127-136.
16. Lee, T.C., M. Oshimura and J.C. Barrett, 1985. Comparison of arsenic-induced cell transformation, cytotoxicity, mutation and cytogenetic effects in Syrian hamster embryo cells in culture. Carcinogenesis, 6: 1421-1426.
17. Zadorozhnaja, T.D., R.E. Little, R.K. Miller, N.A. Mendel, R.J. Taylor, B.J. Presley and B.C. Gladen, 2000. Concentrations of arsenic, cadmium, copper, lead, mercury and zinc in human placentas from two cities in Ukraine. J. Toxicol. Environ. Health, A61: 255-263.
18. Yang, C.Y., C.C. Chang, S.S. Tsai, H.Y. Chuang, C.K. Ho and T.N. Wu, 2003. Arsenic in drinking water and adverse pregnancy outcome in an arseniasis-endemic area in north-eastern Taiwan. Environ. Res., 91: 29-34.
19. hmad, S.A., M.H. Sayed, S. Barua, M.H. Khan, M.H. Faruquee, A. Jalil, S.A. Hadi and H.K. Talukder, 2001. Arsenic in drinking water and pregnancy outcomes. Environ. Health Perspect., 109: 629-631.
20. Goebl, H.H., P.F. Schmidt, J. Bohl, B. Teltenborn, G. Kramer and L. Gutmann, 1990. Polyneuropathy due to arsenic intoxication: Biopsy studies. J. Neurol., 49: 137-149.
21. Neiger, R.D. and G.D. Osweiler, 1989. Effect of subacute low level dietary sodium arsenite on dogs. Fundam. Appl. Toxicol., 13: 439-451.

22. Hei, T.K., Liu Six and C. Waldren, 1998. Mutagenicity of arsenic in mammalian cells: role of reactive oxygen species. Proc. Natl. Acad. Sci., 95: 8103-8107.
23. Hunter, K.W., R.A. Gault and M.D. Berner, 2002. Preparation of micro particulate $-glucan from Saccharomyces cerevisae for use in immune potentiation. Lett. Appl. Microbiol., 35: 267-276.
24. Iyayi, E. and Z. Aderolu, 2004. Enhancement of the feeding value of some agro-industrial by-products for laying hens after their solid state fermentation with Trichoderma viride. Afr. J. Biotechnol., pp: 182-185.
25. D'Amour, F.E. and D.C. Smith, 1965. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther., 72: 74-79.
26. Yadeyi, A.S., F.O. Ajao, A.O. Afolabi, U.S. Udoh and G.F. Ibironke, 2005. Chronic vitamin C administration induces thermal hyperalgesia in male rats. Nig. J. Health Biomed. Sci., 4: 153-155.
27. Oyadeyi, A.S., F.O. Ajao, G.F. Ibironke and A.O. Afolabi, 2005. Acute restraint stress induces hyperalgesia via non-adrenergic mechanisms in rats. Afr. J. Biomed. Res., 8: 123-125.
28. Basbaum, A. and M.C. Bushnell, 2002. Pain: Basic mechanisms. In: Pain 2002-An updated review: Referesher course syllabus, M.A. Giamberardino (ed). IASP press, seattle, pp: 1-2.
29. Sen, C.K., 1995. Oxidants and antioxidants in exercise. J. Appl. Physiol., 79: 675-686.
30. Fillingim, R.B., 2000. Sex, gender and pain: A biopsychosocial framework. In: Sex, Gender and Pain, R.B. Fillingim (ed). IASP Pres, Seattle, pp: 1-5.
31. Aloisi, A.M., 2000. Sensory effects of gonadal hormones. In: Sex, Gender and Pain, R.B. Fillingim (ed). IASP Press, Seattle, pp: 7-24.
32. Fillingim, R.B. and T.J. Ness, 2000. The influence of menstrual cycle and sex hormones on pain responses in humans. In: Sex, Gender and Pain, R.B. Fillingim (ed). IASP press, Seattle, pp: 191-207.
33. Robinson, M.E., J.L. Riley III and C.D. Myers, 2000. Psychosocial Contributions to sex-related differences in pain responses. In: Sex, Gender and Pain, R.B. Fillingim (ed). IASP press, Seattle, pp: 41-68.
34. Icero, T.J., B. Nock and E.R. Meyer, 1996. Gender-related differences in the antinociceptive properties of morphine. J. Pharmacol. Exp. Ther., 279: 767-773.
35. Boyer, J.S., M.M. Morgan and R.M. Craft, 1998. Microinjection of morphine into the rostral ventromedial medulla produces greater antinociception in male compared to female rats. Brain Res., 796: 315-318.
36. Ofunya, C.O. and C.J. Nwanjiuba, 1990. Microbial degradation and utilization of cassava peel. World J. Microbiol. Biotechnol., 6: 144-146.
37. Iyayi, E.A. and D.M. Losel, 2001. Changes in the carbohydrate fractions of cassava peels following fungal solid state fermentation. J. Food Technol. Africa, 6: 101-103.
38. Lull, C., H.J. Wichers and H.F.B. Savel Koul, 2005. Anti-inflammatory and immunomodulating properties of fungal metabolites. Mediators Inflamm,. pp: 63-80.

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 90-98, 2007
ISSN 1818-6769

Performance and Some Blood Chemistry Indices of Broiler Chicken Served Fluted Pumpkin (*Telfaria occidentalis*) Leaves Extract Supplement

F.C. Nworgu, S.A. Ogungbenro and K.S. Solesi

Federal College of Animal Health and Production Technology,
Institute of Agricultural Research and Training, Obafemi Awolowo University,
Moor Plantation, P.M.B. 5029, Ibadan, Nigeria

Abstract: A 56-day experiment involving 120 day-old Anak 2000 broiler chicks was carried out in a completely randomized design to evaluate the performance, haematological parameters and serum metabolites of the broilers served Fluted Pumpkin Leaves Extract (FPLE) supplement at four days interval for 8 weeks during the late dry season. The birds were allotted to 5 treatments containing 0, 30, 60, 90 and 120 ml FPLE/litre of water. Each treatment was replicated three times. Broiler starters were fed the same starter diet, while the finishers were equally fed the same finisher diet. The FPLE was found to be rich in protein (21.31%) and ash (10.97%) most especially Ca, P, Mg and Fe and relatively low in fibre, tannin and oxalate, hence a good protein and mineral supplement for broilers during the late dry season. Feed intake, body weight gain, feed conversion ratio, water intake, cost of fed per kg live weight gain, haemoglobin, Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), Cholesterol (CH), urea, sodium and potassium of the birds served FPLE were significantly ($p<0.05$) superior to control. Haemoglobin was elevated on the birds served PFLE by 19.64-48.2% compare to control. The birds served 60 ml FPLE/litre of water had elevated Hb, PCV and RBC, while those on 120 ml had the highest value of WBC, CH and urea. Broiler starters and finishers are recommended to be served 60 and 120 ml FPLE/litre of water, respectively at 4 days interval during late dry season for improved feed intake, weight gain and blood formation.

Key words: Performance % haematology % serum biochemistry % broiler chicken % fluted pumpin leaves extract % supplement

INTRODUCTION

Rapid growth of human and livestock population which has created increased needs for food and feed in the developing countries demand that alternative feed resources must be identified and evaluated. More also, the scarcity of locally produced protein supplements for animal diets in the tropics has created a need to finding alternative feed sources. Hence, widely cultivated vegetable in the tropics and sub-tropices known as Fluted Pumpkin (FP) (*Telfaria occidentalis*) needs to be turned attention towards exploitation its leaves extract as protein and mineral supplements in poultry nutrition. Leafy vegetables supply minerals, protein and vitamins, thereby complementing the inadequacies of most feedstuffs [1]. The protein from leaves may be recovered and fed animals as solution in form of protein concentrates [2]. In Nigeria, *T. occidentalis* leaves extract is regarded as blood tonic for both the rich and the poor [3]. Adedapo *et al*. [4] used FPand sorghum bicolor extracts as potent haematnics in domestic rabbits and concluded that the rabbits served these extracts had the highest values of packed cell volume, haemoglobin, red blood and white blood cells and faster responded to therapy. The use of *T. occidentalis* leaves extract supplement in poultry nutrition is not common in our environment. The nutritive value of the leaves of FP has been evaluated chemically and are found to contain (g/100 g DM) 30.5±2.5 crude protein, 3.0±0.15 crude lipid, 8.3±50 crude fibre and 8.4±0.5 total ash [5]. The authors noted that the leaves had low level of tannic acid (4.75±0.50 mg 100 g DM) and oxalate (0.45±0.03 mg/100 g DM) but high level of phytic acid (20.5±2.10 mg/100 g DM).

Corresponding Author: Dr. F.C. Nworgu, Federal College of Animal Health and Production Technology, Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, P.M.B. 5029, Ibadan, Nigeria

Idufueko [6] and Madubuike [7] reported that poultry meat and eggs offer considerable potential for bridging the protein gap in view of the fact that high yielding exotic poultry are easily adaptable to our environment and the technology of production is relatively simple with returns on investment appreciably high. High cost of feed in poultry industry in Nigeria is the major problem of poultry farmers [8]. Opara [9] reported that feed accounts for 70-85% of the total production cost of poultry in Nigeria. More than 50% of the Nigerian poultry farms have closed down and another 30% are forced to reduce their production capacity due to high cost and shortage of feed [10].

Blood parameters have been shown to be major indices of physiological, pathological and nutritional status of an organism and changes in the constituent compounds of blood when compared to normal values could be used to interpret the metabolic stage of an animal as well as quality of feed [11]. It is against these backgrounds that this study was conducted to determine the nutritive value of *T. occidentalis* leaves extract and evaluate its effects on the weight gain, feed and water intake, haematology and serum biochemistry indices of broiler chickens served this extract at four days interval during the late dry season (December 2003-February, 2004) as birds loose weight during this period of the year due to positive heat load.

MATERIALS AND METHODS

A total of one hundred and twenty Anak 2000 day-old broiler chicks were weighed and randomly allotted to five dietary treatments containing 0, 30, 60, 90 and 120 ml of fluted pumpkin leaves extract (FPLE) per one litre of water for A, B, C, D and E correspondingly in a Completely Randomized Design. Treatment A served as control. Each treatment was replicated three times with 8 birds per replicate. The experiment lasted for eight weeks (i.e. [4] for weeks for each phase). The broiler starters were fed the same starter diet, while broilers finishers were equally fed the same finisher diet (Table 1). The birds were served the FPLE according to the treatments per one litre of water and later water was served freely. Feed and water were served *ad-libitum*. The FPLE was served at four days interval throughout the period of the experiment. Data on feed and water intake were recorded on daily basis, while weight gain was determined on weekly basis and feed conversion ratio was calculated at the end of each phase. Other management practices such as routine vaccination, drug administration and maintenance of cleanliness within and outside the poultry pens/house were observed.

Table 1: Gross composition of broiler chicken diets

Ingredients (%)	Broiler starter diet	Broiler finisher diet
Maize	48.00	50.00
Corn bran	6.85	8.00
Palm kernel cake	5.00	8.10
Soybean meal	20.00	17.00
Groundnut cake	12.00	9.00
Fish meal (65%)	4.20	3.50
Bone meal	3.00	3.50
Vitamin and mineral premix*	0.30	0.35
Salt	0.25	0.25
Lysine	0.25	0.20
Methionine	0.15	0.10
Calculated analysis (%)		
Crude protein	23.07	21.00
Crude fibre	3.42	4.07
Lysine	1.20	0.88
Methionine	0.62	0.50
Metabolisable energy (kcal kg^{-1})	2996.00	3100.00

*To provide the following per (kg) of diet: Vit A = 10,000iu, vitamin D3 = 2000 iu, vitamin E = 5 iu, vitamin K = 2 mg, riboflavin = 4.20 mg, vitamin B 12 = 0.01 mg, pantothenic acid = 5 mg, nicotinic acid = 20 mg, folic acid = 0.5 mg, choline = 3 mg, Mg = 56 mg, Fe = 20 mg, Cu = 10 mg, Zn = 50 mg, Co = 125 mg and Iodine = 0.08 mg

Preparation of fluted pumpkin leaves extract: One kilogramme of freshly cut fluted pumpkin leaves with leaf stalks were washed, drained, chopped and pounded in a mortar with pestle. This was then squeezed and filtered with a sieve to obtain the homogenous extract of the Fluted Pumpkin Leaves (FPL). The homogenous FPLE was prepared at four days interval and served the animals fresh according to the treatments.

Collection of blood samples: Blood collection was carried out at the 8th week of the experiment. Three birds per treatment were randomly selected and bled via wing veins using sterile gauge 19 needles and syringes. About 5 ml of blood was collected into two sets of three sterilized glass tubes/bottles. For haematology, the blood samples were collected into two sets of three sterilized bottles containing Ethylene Diaminetetra-acetic Acid (EDTA). Blood samples for serum biochemical studies were collected into plain vacutainers (i.e. without anticoagulant) for serum separation. Serum was obtained

by centrifugation and the serum samples were stored in a deep freezer (at minus 10°C) until analyzed.

Analysis of blood samples: Packed Cell Volume (PCV) was determined by microhaematocrit method [12]. Haemoglobin (Hb) concentration was measured spectrophotometrically by cyanomethaemoglobin method [13] and [12] using SP6-500 UV spectrophotometer (Pye UNICAM ENGLAND). The Red Blood Cell (RBC) and white blood cell counts were estimated using haemocytometer [12]. Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) were calculated from Hb, PVC and RBC [14]. Serum Total Protein (STP) was determined by Kjedahl method as described by Kohn and Allen [15], while albumin was determined using the BCG (bromocresol green) method as described by Peters *et al.* [16]. Aspartate Transaminase (AST) and Alanine Transaminase (ALT) activities were determined using spectrophotometric method as described by Rej and Holder [17] and Holder and Rej [18], respectively.

Sodium and potassium were determined by flame photometry [19]. Cholesterol was determined according to Roschlan *et al*. [20], while urea was determined as described by Kaplan and Szabo [21].

Proximate and chemical analyses: Proximate and chemical composition of the feeds and the FPLE were determined According to the Official Method of Analysis (AOAC) [22]. The minerals were determined by the procedures outlined by Boehringer [23] and AOAC [22]. Sodium and calcium were read with PFP7 flame photometer and phosphorus was determined with spectrotometer (spectronic21). Gross energy values were determined using the bomb calorimeter method [24], while metabolizable energy was estimated by the method outlined by Panzenga [25]. Phytate was determined by the technique of Igbedion *et al*. [26], while tannin was determined by the procedures outlined by Hagerman and Ler [27] and oxalate by the method of Talapatra and Price [28].

Statistical analysis: Data collected were subjected to Analysis Variance (ANOVA) in SPSS 10 computer programme and errors were calculated as Standard Errors of the Mean (SEM). Significant treatment means were compared using Duncan's New Multiple Range Test as outlined by Obi [29]. Significance was accepted at the 0.5 level of probability.

Table 2: Proximate chemical composition of fluted pumpkin leaf extract (FPLE) and broiler chicken diets (%DM basis)

Fraction	Fluted pumpkin leaf extract	Broiler starter diet	Broiler finisher diet
Crude protein	21.31	22.12	21.00
Crude fibre	6.41	3.50	3.48
Ether extract	5.50	3.46	3.84
Ash	10.92	7.00	6.11
Nitrogen free extract	55.56	62.92	65.57
Metabolizable energy (Kca kgG^{1})*	3121.00	3072.00	3118.00
Gross energy (kcal kgG^{1})	4420.00	-	-
Calcium	0.67	0.96	0.80
Phosphorus	0.40	0.40	0.34
Potassium	0.15	1.28	1.31
Nitrogen	3.41	3.70	3.36
Magnesium	0.43	0.26	0.21
Sodium	0.02	0.26	0.28
Zinc (mg/100 g DM)	7.50	4.8	5.10
Iron (mg/100g/DM)	18.50	1.14	1.20
Manganese (mg/100 g DM)	1.18	7.90	9.20
Phytate (mg/100 g DM)	510.51	-	-
Tannin (mg/100 g DM)	0.184	-	-
Oxalate (mg/100 g DM)	0.0034	-	-

* Determined by Pazenga [25]

RESULTS AND DISCUSSION

The proximate chemical compositions of Fluted Pumpkin Leaves Extract (FPLE), broiler starter and finisher diets are presented in Table 2. The broiler chicken diets presented here met the requirements of the birds and are in line with NRC [30] standards. The FPLE is a valuable feed supplement for broilers, especially during the late dry season of the year, being very rich in Crude Protein (CP) (21.31%), minerals (calcium, phosphorus, magnesium and iron) and relatively low in crude fibre (5.5%), oxalate and tannin. The CP value of FPLE in this study agrees with the values reported by Okoli and Mgbeogba [31] (21.8%) and Akwaowo *et al*. [32] (22.4%), but lower than the report of Ladeji *et al*. [33] (30.5%). The crude fibre (5.5%) in this study is lower than the results of Akwaowo *et al*. [32] (10.10%) and Ladeji *et al*. [33] (8.5%). The ash (10.92%) content of FPLE in the present study is in line with the findings of Ladeji *et al*. [33] and Akwaowo *et al*. [32] whose values were 8.40 and 12.60%, respectively. The values of minerals reported in the study do not corroborate with the findings of Akaowo *et al*. [32], whose values were lower. The values (mg/100 g DM) of iron (12.0), calcium (144.0) and magnesium (100) reported

Table 3: Performance characteristics of broiler chicken served Fluted Pumpkin Leaves Extracts (FPLE) and experimental diets

	Starter phase					
Parameters	A (0 ml l G^{1})	B (30 ml l G^{1})	C (60 ml l G^{1})	D (90 ml l G^{1})	E (120 ml l G^{1})	SEM
Initial body weight (g/bird)	44.00	44.01	44.00	44.02	44.01	-
Final body weight (g/bird)	535.71^{c}	528.57^{c}	576.19^{a}	562.38^{b}	530.95^{c}	3.92
Mean body weight gain (g/bird)	491.71^{c}	484.56^{d}	532.19^{a}	518.36^{b}	486.94^{cd}	1.96
Average daily weight gain (g/bird)	17.56	17.30	19.00	18.51	17.39	-
Total feed intake (g/bird)	1100.60^{b}	1099.11^{c}	1067.90^{d}	1068.91^{d}	1108.80^{a}	0.13
Average daily feed intake (g/bird)	39.30	39.25	38.13	38.17	39.59	-
Feed conversion ratio (feed/g grain)	2.24^{b}	2.27^{a}	2.01^{d}	2.06^{c}	2.28^{a}	0.006
Total water intake (ml/bird)	1823.90^{c}	1850.21^{b}	1799.00^{d}	1775.30^{e}	1887.00^{a}	0.29
Average daily water intake(ml/bird)	65.14	66.08	64.30	63.33	67.41	-
Feed water intake ratio	01:02.00	01:01.70	01:01.70	01:01.70	01:01.70	-
Mortality (%)	-	-	-	-	-	-
Cost of feed per kg live weight gain (N/kg)	92.81^{a}	94.06^{a}	83.22^{b}	85.52^{b}	94.41^{a}	1.15
Cost of FPLE (N/bird)	-	1.3	2.6	3.9	5.2	-
	Finisher phase					
Parameters	A (0 ml l G^{1})	B (30 ml l G^{1})	C (60 ml l G^{1})	D (90 ml l G^{1})	E (120 ml l G^{1})	SEM
Initial body weight (g/bird)	535.71^{c}	528.57^{c}	576.19^{a}	562.38^{b}	530.95^{c}	3.92
Final body weight (g/bird)	1720.19^{e}	1951.40^{b}	1882.09^{d}	1888.70^{c}	2133.70^{a}	1.29
Mean body weight gain (g/bird)	1184.48^{e}	1422.57^{b}	1305.90^{d}	1326.32^{c}	1602.03^{d}	1.59
Average daily weight gain (g/bird)	42.30	50.81	46.64	47.37	57.24	-
Total feed intake (g/bird)	3063.70^{d}	3031.32^{e}	3210.86^{c}	3235.60^{b}	3333.61^{a}	5.09
Average daily feed intake (g/bird)	109.41	108.46	114.67	115.55	119.05	-
Feed conversion ratio (feed/g grain)	2.59^{a}	2.13^{d}	2.46^{b}	2.44^{c}	2.02^{e}	0.001
Total water intake (ml/bird)	8835.14^{e}	8968.40^{d}	9109.86^{b}	9072.30^{c}	9174.68^{a}	0.08
Average daily water intake(ml/bird)	313.54	320.30	325.35	324.01	327.67	-
Feed water intake ratio	01:02.90	01:03.00	01:02.80	01:02.80	01:02.80	-
Mortality (%)	-	-	-	4.70	4.70	-
Cost of feed per kg live weight gain (N/kg)	100.23^{a}	82.56^{d}	95.25^{b}	94.52^{c}	80.59^{e}	0.06
Cost of FPLE (N/bird)	-	1.30	2.60	3.90	5.20	-

abcd = Means with different superscripts on the same horizontal row within each phase differ significantly ($p<0.05$), FPLE = Fluted pumpkin leaves extract, A = Oml FPLE/l of water, B = 30ml FPLE/l of H_2O, C = 60ml FPLE/l of H_2O, D = 90ml FPLE/l of H_2O and E = 120ml FPLE/l of H_2O, SEM =Standard error of means, N = Naira

by Ladeji *et al.* [33] do not concur with the present study, unlike iron (5.0) and potassium (594). Tannin and oxalate (mg/100 g DM) values is this study agree with the study of Akwaowo *et al.* [32] and Ladeji *et al.* [33], unlike phytate which has elevated value in the present study. Variations in these values could be attributed to age of cutting, variety, season of planting and agronomic practical adopted.

The Final Body Weight (FBW), Weight Gain (WG), Feed Intake (FI), Total Water Intake (TWI), Feed Conversion Ratio (FCR) and Cost of Feed per Kilogramme Live Weight Gain (CFPKLWG) were significantly ($p<0.05$)different among the treatments (Table 3). At starter phase the best WG (532.19 g/bird) was observed on the birds served 60 ml FPLE/litre of water compared to control and 120 ml FPLE/L H_2O (486.94-491.719 g/bird), but at finisher's phase, the birds served 120 ml FPLE had the best result for FBW (2133.70 g/bird) unlike control (1720.19 g/bird). This indicates that broiler starters tolerated lower concentration of FPLE, while broiler

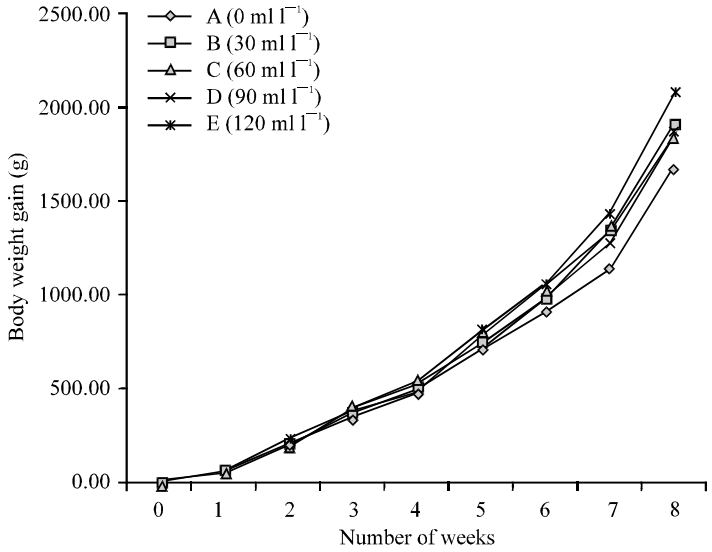

Fig. 1: Effect of fluted pumpkin leaves extract on body weight gain of broiler checked

finishers tolerated higher concentration. This is likely due to their better developed organs. In both phases, the highest F1 was recorded on the birds served 120 ml FPLE/litre H_2O. This could be as a result of availability of minerals which improved feed intake. Oluyemi and Roberts [34] reported that both micro and macro elements improve F1 in poultry. The TWI was similarly highest at this concentration in both phases. The TWI here agrees with the report of Oluyemi and Roberts [34]. The WG for birds served 60 ml FPLE/litre H_2O (532.19 g/bird) and those in control (491.71 g/bird) corroborate with the report of Nworgu and Egbunike [35] (259.0-586.0 g/bird) and at finisher's phase the WG (1184.48-1602.03 g/bird) agrees with the values reported by Esonu *et al.* [36] (982.0-1405.0 g/bird). The FCR for broiler starters is lower (better) than that reported by Odunsi *et al.* [37] (2.59-2.88) when the authors fed broiler chicks wild sunflower leaves meal, while the FCR at finisher phase was better than the record of Esonu *et al*. [36] (3.60-4.66). The CFPKWG for broiler starters is not in harmony with the result of Nowrgu *et al*. [38] (N53.39-N54.96 /kg)), while such parameter is in agreement with these authors (94.92-N106.36 /kg) at finisher phase. Variation at starters phase for FCR was as a result of poor weight gain in this study compared to Nworgu *et al*. [38] (671.2-746.0 g/bird) when the birds were fed full fat soybean and soybean meal. Mortality recorded was as a result of coccidiosis infection. Figure 1 shows the weight gain curves of the birds in each treatment. It reveals that birds served FPLE gained more weight than those in control.

The Haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) and Mean Corpuscular Volume (MCV) were highly significant ($p<0.05$) and increased in the birds served FPLE compare to control (Table 4). The best Hb (10.0%), PCV (41.50%) and WBC ($3.39x\ 10^6$/l) were recorded on the birds served 60 ml/FPLE, while the least of these parameters (7.66, 28.00 and $2.35\ x\ 10^6$ /l, respectively) was recorded in control. This indicates that FPLE helps in blood formation, mostly at 60 ml/litre of H_2O, due to availability of protein, iron, calcium, magnesium, potassium and phosphorus as the birds ate more feed when they were served with FPLE. This is likely one of the reasons in Nigeria why anaemic patients, pregnant women, men and children are advised by medical personnels to take FPLE mixed with milk or honey or sole. The values of Hb, PCV, MCHC in this study are in harmony with the reports of Iheukwumene and Herbert [39] whose values were 6.0-13.0%, 29.0-38.0% and 33.0-35.0 pg, respectively. Islam *et al*. [40] reported that commercial and local chicken reared in Sylhet region in Bangladesh had Hb value of 7.06-9.37%, PCV value of 26.56-34.60% and MCV value of 84.27-163.56 fl and the values of these parameters are in line with the report of this study. The Hb, PCV, MCV, MCHC and RBC reported in the present study agree with that reported by MVM [41] for chicken whose values were 9-13%, 30-40%, 127 fl, 29% and $3.0x10^6$ /l, respectively. Awotwi [42] reported that PCV of local and commercial chickens in Ghana varied from 32.88-33.20 and 31.30-35.60%, respectively, while Nworgu *et al.* [43] reported PCV of 28-30.0% for cockerel chicks fed cocoa pod husk meal.

The Total Serum Protein (TSP), albumin, cholesterol (CH), urea, Alamine Transaminase (ALT), Aspartate Transaminase (AST), sodium and potassium were

Table 4: Haematological parameters of broilers served Fluted Pumpkin Leaves Extract (FPLE) supplement

	Treatments					
Parameters	A (0 ml l G^1)	B (30 ml l G^1)	C (60 ml l G^1)	D (90 ml l G^1)	E (120 ml l G^1)	SEM
Haemoglobin (gm%)	7.66^e	9.59^b	10.00^a	8.59^d	8.92^c	0.01
Packed cell volume (%)	28.00^d	36.50^b	41.50^a	33.50^c	36.50^b	0.02
Red blood cell (x10^6/l)	2.35^d	2.83^c	3.30^a	2.38^d	3.23^b	0.01
White blood cell (x10^6/l)	2.17^d	2.30^c	2.92^b	2.08^e	3.48^a	0.01
Mean corpuscular/cell volume (fl)	119.40^d	128.97^b	122.41^c	140.75^a	97.85^e	0.05
Mean cell haemoglobin (pg/cell)	32.59^c	33.88^b	29.49^d	36.09^a	23.91^e	0.02
Mean cell haemoglobin concentration (%)	27.35^a	26.27^b	24.09^e	25.64^c	24.34^d	0.02

Table 5: Serum metabolite indices of broiler chicken served fluted pumpkin leaves extract (FPLE) supplement

	Treatments					
Parameters	A (O ml l G^1)	B (30 ml l G^1)	C (60 ml l G^1)	D (90 ml l G^1)	E (120 ml l G^1)	SEM
Total serum protein (g dlG^1)	3.50^b	3.70^a	3.50^b	3.60^{ab}	3.10^c	0.06
Albumin (g dlG^1)	2.10^b	2.40^a	2.40^a	2.30^{ab}	2.00^b	0.09
Globulin (g dlG^1)	1.40^a	1.30^{ab}	1.10^b	1.30^{ab}	1.10^b	0.01
Albumin globulin ratio	1.50^c	1.80^b	2.20^a	1.80^b	1.80^b	0.09
Cholesterol (mg dlG^1)	143.10^b	147.00^b	150.12^b	158.10^{ab}	163.00^a	0.61
Urea (mg dlG^1)	14.00^c	12.01^d	10.01^e	17.00^b	19.00^a	0.35
Alanine transaminase (iu lG^1)	23.50	23.50	23.00	24.00	24.84	0.26
Aspartate transaminase (iu lG^1)	19.00^b	19.00^b	17.00^c	21.11^a	19.00^b	0.32
Sodium (mmol lG^1)	103.10^e	112.00^d	121.00^b	117.00^c	131.00^a	1.10
Potassium (mmol lG^1)	4.40^b	4.80^a	5.10^a	5.20^a	5.30^a	0.06

abcde: Means with different superscripts on the same horizontal row differ significantly ($p<0.05$)

significantly ($p<0.05$) improved on the birds served FPLE compared to control (Table 5). The values of urea, sodium and potassium in this study are higher than the report of Iheukwumene and Herbert [39], while results of Nworgu [8] for TSP (6.50-6.77 g dlG^1) and urea (21.01-24.00 mg dlG^1) are higher than reported in the present study. However, the values of sodium, potassium, ALT and AST in this study are in line with the submission of Nworgu [8] when broilers were fed mimosa leaf meal. The value of CH reported by Aderemi [44] (100.30-108.21 mg dlG^1) and Nworgu [8] (93.33-116.67 mg dlG^1) is lower than reported in this study. Variations in CH could be attributed to breed of chicken, nutritional pattern, type of feed, environmental factors and the test ingredient used. The CH in this study agrees with the values reported by Sturkie *et al.* [45] (100-150 mg dlG^1). Highest value of CH for birds severed 90-120 ml FPLE in this study could be attributed to low fibre content of the FPLE which could not have a binding effect on the bile acids excreting such and thus resulted in higher level of serum cholesterol. When the scenario above is on, recycling of bile acid is increased thereby synthesis of more bile acid from CH is not stimulated resulting again in higher level of CH. This is in agreement with the findings of Ezeagu *et al.* [46]. Matawalli *et al.* [47] conducted experiment on the effect of methanolic leaf extent of *Adansonia digitata* on serum lipid levels in normal and ethanol fed rats and reported that the extract lowered the lipid levels in rat fed with alcohol and adduced/concluded that the possible hypolipiaemic effect could be attributed to the presence of saponins and fibre in the extract, which has been shown to bind to serum lipids especially CH, thereby easing their excretion from circulation. Sturike *et al.* [45] noted that an increase in the CH level of the feed of an animal, consequently would lead to an increase in the CH level of the blood of the animal and the authors reported that the value of CH in chicken varies from 100-150 mg dlG^1. Higher values of CH depicts hyperlipeamia indicating that the patient is likely to have heart disease. The TSP in study was maintained by slight increase of albumin fraction with corresponding decrease in globulin fraction. Harper [48] highlighted that increased in serum globulin in infected animal is expected since this fraction of protein is the principal site of the circulating antibodies

(immunoglobulins). Egyum [49] and Iyayi and Tewe [50] reported that serum urea and TSP depend on both the quality and the quantity of the protein supplied in the diet. Higher level of urea for the birds fed 120 ml FPLE/litre of H_2O could be attributed to the presence of some anti-nutritional factors which might have lowered the quality of the protein indicating imbalance of amino acids in the diet which caused elevated blood urea concentration [51]. However, kidney malformation may raise the level of blood urea. The values of ALT and AST in this study are similar to the reports of Nworgu [8]. Positive correlation between ALT and growth performance, protein quality and quantity of the diet was reported by Balogun [52]. Higher values of sodium and potassium reported in the birds served FPLE reveals that FPLE are rich in these minerals. Value of potassium reported here is similar to the values reported by Nworgu [8] (3.87-4.37 mmol l^{-1}), while value of sodium is higher than the submission of Iheukuwemene and Herbert [39].

CONCLUSIONS

The FPLE is rich in protein and minerals and low in fibre, tannin and oxalate. The FPLE is a valuable protein and mineral supplement for broiler chicken during the dry season in the tropics, as it encouraged feed and water intake, weight gain and blood formation. Birds severed FPLE had increased weight gain, Hb, PCV, RBC, serum sodium and potassium which were 10.52-35.25, 12.14-30.55, 19.64-48.21, 1.28-41.28, 8.63-26.06 and 9.09-20.45%, respectively with respect to control in 8 weeks. Broiler finishers tolerated higher concentration of the FPLE than broiler starters. Hence, 60 and 120 ml FPLE/litre of water are recommended for broiler starters and finishers, respectively.

REFERENCES

1. Ifon, E.T. and O. Basir, 1980. The nutritive value of some Nigerian leafy vegetables, particularly vitamin and mineral contents. Food Chem., 4: 263-267.
2. Farinu, G.O., S.O. Ajiboye and S. Ajao, 1992. Chemical composition and nutritive value of leaf protein concentrate from *Leuaena leucocephala.* J. Sci. Food Agric., 59: 127-129.
3. Egbunike, G.N. and F.C. Nworgu, 2005. Traditional applications to livestock management and improvement in Nigeria. An invited paper presented at the 2nd Ann. Conf. Nigerian Society of Indigenous Knowledge and Development (NSIKAD) at Cross River University of Technology, Obubra Campus on the 9-11th November, pp: 1-25.
4. Adedapo, A.A., O.A. Dina, A.B. Saba and O.D. Oladipo, 2002. Evaluation of *Telfaria occidentalis* and *Sorghum bicolo*r extracts as potent haematinics in domestic rabbits. Nig. J. Anim. Prod., 29: 88-93.
5. Ladeji, O., Z.S.C. Okoye and T. Ojebe, 1995. Chemical evaluation of the nutritive value of leaf of fluted pumpkin (*Telfaria occidentalis*). Food Chem., 53: 353-355.
6. Idufueko, A., 1984. Self-sufficiency in animal protein supply under changing economic fortunes. Nig. J. Anim. Prod., 11: 14-21.
7. Madubuike, F.N., 1992. Bridging the animal protein gap for rural development in Nigeria. The potential of pigs. J. Agric. Rural Develop., 5: 5-12.
8. Nworgu, F.C., 2004. Utilization of forage meal supplements in broiler production Ph.D. Thesis. Animal Science Dept. University of Ibadan, Ibadan Nigeria, pp: 214-222.
9. Opara, C.C., 1996. Studies on the use of *Alchornia cordifolia* leaf meal as feed ingredient in poultry diets. M.Sc. Thesis. Federal University of Technology, Owerri, Nigeria, pp: 150-159.
10. Esonu, B.O., O.O. Emenalom, A.B.I. Udedibie, U. Herbert, C.F. Ekpor, I.C. Okoli and F.C. Iheukwumere, 2001. Performance and blood chemistry of weaner pigs fed raw Mucuna (*Velvet bean*) meal. Trop. Anim. Prod. Investigation, 4: 49-54.
11. Babatunde, G.M., A.O. Fajimi and A.O. Oyejide, 1992. Rubber seed oil versus palm oil in broiler chicken diets. Effects on perfermance, nutrient digestibility, haemotology and carcass characteristics. Anim. Feed Sci. Technol., 35: 133-146.
12. Schalm, J.W., N.C. Jain and E.J. Carol, 1975. Veterinary Haematology. 3rd Edn. Lea and Febriger. Philadelphia USA, pp: 15-81.
13. Swensen, M.T., 1951. Effects of Vitamin B12 and liver meal on the haematology of chicks fed on low protein ration. Anim. J. Vet. Res., 12: 147-151.
14. Jain, N.C., 1986. Schalm's Veterinary Haematology. 4th Edn. Lea and Febrigen, Philadelphia, USA., pp: 34-50.
15. Kohn, R.A. and M.S. Allen, 1995. Enrichment of proteolysis activity relative to nitrogen in preparations from the rumen for *in vitro* studies. Anim. Feed Sci. Technol., pp: 52: 1-4.
16. Peters, T., G.T. Biamonte and B.T. Doumas, 1982. Protein (total protein) in serum. In: Selected methods of clinical chemistry. Faulkner, G.W.R. and S. Mcites (Eds.). Am. Assoc. Clin. Chem., pp: 100-115.
17. Rej, R. and M. Holder, 1983. Aspratate aminotransferase. In: Methods of enzymatic analysis. 3rd Edn., Bergmeyer, H.U., J. Bergmeyer and M. Grassl (Eds.). Weinhein Velagchemie, pp: 416-433.

18. Holder, M. and R. Rej, 1983. Alanine transaminase. In: Methods of enzymatics analysis. 3rd Edn. Bergmeyer, H.U., J. Bergmeyer and M. Gassl (Eds.). Weinhein Velagchemie, pp: 380-401.
19. Mouldin, R.E., O.A. Goldale, R.M. Engeman, M.J. Goodall, R.K. Craves and J.J. Johnston, 1996. Determination of zinc phoshide residue in the California ground squirrel (*Speraphilus beecheyi*) by gas chromatography flame photometric determination. J. Agric. Food Chem., 11: 189-191.
20. Roschlan, P., E. Bernet and W. Guber, 1974. Enzymatische bestimmung des gesant cholesterium in serum. J. Clin. Biochem., 12: 403-407.
21. Kaplan, A. and I.I. Szabo, 1979. Clinical Chemistry: Interpretation and technique. 1st Edn. Henry Kumpton Publishers, London, pp: 109-110.
22. AOAC., 1990. Association of Official Analytical Chemists. Official methods of analysis. 15th Edn. Washington D.C.
23. Boehringer Mannheim GmbH Diagnostica, 1979. 6800 Mannheim 31, Germany Cat. No. 124974.
24. Miller, D.S. and P.R. Payne, 1959. A ballistic bomb calorimeter. Br. J. Nutr., 13: 501-508.
25. Panzenga, U., 1985. Feeding parent stock. Zootecncia International, pp: 22-24.
26. Igbedioh, S.O., K.T.A. Olugbemi and M.A. Akpapunam, 1994. Effect of processing methods on phytic acid level in bambara nut and pigeon pea. Food Chem., 50: 147-151.
27. Hagerman, A.E. and I.G Ler, 1983. Precipitation methods for the qualitative determination of tannis. J. Agric. Food Chem., 26: 809-812.
28. Talapatra, E.L. and B.L. Price, 1948. Oxalate: Its chemistry and nutritional signficance. World Review of Nutrition and Dietetics. Krager, New York.
29. Obi, I.U., 1990. Statistical methods of detecting differences between treatment means. 2nd Edn. Snaap Press, Enugu, Nigeria, pp: 25-85.
30. NRC., 1994. National Research Council. Nutrient requirement of poultry. 9th Rev. Edn. Washington DC., USA.
31. Okoli, B.E. and C.M. Mgbeogba, 1983. Fluted pumpkin (*Telfairia occidentalis*), West African Vegetable Crop. Econ. Bot., 37: 145-149.
32. Akwaowo, E.U., B.A. Ndon and E.U. Etuk, 2000. Minerals and anti-nutrients in fluted pumpkin (*Telfairia occidentalis* Hook f.). Food Chem., 70: 235-240.
33. Ladeji, O., Z.S.C. Okoye and T. Ojobe, 1995. Chemical evaluation of the nutritive value of leaf of fluted pumpkin (*Telfairia occidentalis*). Food Chem., 53: 353-355.
34. Oluyemi, J.A. and F.A. Roberts, 2000. Poultry Production in warm climate. Spectrum Books Limited. 2nd Rev. Edn., pp: 140-153.
35. Nworgu, F.C. and G.N. Egbunike, 1999. Performance characteristics of broilers fed diets with soyabean meal and groundnut cake as protein sources. Trop. Anim. Prod. Investigation, 2: 51-59.
36. Esonu, B.O., F.C. Iheukwumere, O.O. Emenalom, M.C. Uchegbu and E.B. Etuk, 2002. Performance, nutrient utilization and organ characteristics of broilers fed *Microdesmis puberula* leaf meal. Livestock Research for Rural Development, Vol: 14.
37. Odunsi, A.A., G.O. Farinu, J.O. Akinola and V.A. Togun, 1999. Growth, Carcass characteristics and body composition of broiler chicks fed wild sunflower (*Tithonia diversifolia*) forage meal. Trop. Anim. Prod. Investigation, 2: 205-211.
38. Nworgu, F.C., G.N. Egbunike, F.I. Ogundola, R.A. Salako and O.E. Fakeye, 2001. Effects of different dietary protein levels on the performance and carcass characheristics of broilers ASSET Series A., I: 75-87.
39. Iheukwumere, F.C. and H. Herbert, 2003. Physiological of broiler chickens to quantitative water restrictions: Haematology and serum biochemistry. Intl. J. Poul. Sci., 2: 117-119.
40. Islam, M.S., N.S. Lucky, M.R. Islam, A. Ahad, B.R. Das, M.M. Rahman and M.S.I. Siddiui, 2004. Haematology parameters of Fayumi, Assil and local chikens reared in Sylhet Region in Bangladesh. Intl. J. Poul. Sci., 3: 144-147.
41. MVM., 1986. The Merck Veterinary Manual. 6th Edn. Merck and Co., INC. Rahway, N.J., USA, pp: 1-19.
42. Awotwi, E.K., 1990. A study of baseline haematological value of domestic and commercial chickens in Ghana. Bulletin Animal Production Africa, 38: 453-458.
43. Nworgu, F.C., R.A. Hamzat, O.A. Oduola and M.A. Arasi, 2003. Performance and some haematological indices of cockerel chicks fed cocoa (*Theobroma cacao*) pod husk. ASSET Series A, 3: 11-21.
44. Aderemi, F.A., 2004. Effects of replacement of wheat bran with cassava root sieviate supplemented or unsupplemented with enzyme on the haematology and serum biochemistry of pullet chicks. Trop. J. Anim. Sci., 7: 147-153.

45. Sturkie, P.O., W. Hazel and R. Wood, 2000. Avian Physiology. 3rd Edn. New York. NY Springer-Vallock, New York, pp: 54-60.
46. Ezeagu, I.E., J.J. Petzke., A.D. Ologhobo, C.C. Metges and A.O. Akinsoyinu, 2000. Compositional evaluation and feed potential of the African Kudzu (*Pueraria phaseoloides*, Roxb. Benth) seed meal. J. Food Sci. Technol., 37: 191-195.
47. Matawalli, A.G., A.C. Samuel and S. Yagana, 2004. Effects of methanolic leaf extract of *Adansonia digitata* on serum lipid levels in normal and ethanol fed rats. Pak. J. Biol. Sci., 7: 1094-1095.
48. Harper, H.A., 1982. Review of physiological chemistry. 15th Edn. Lange Medical Publications, pp: 553.
49. Egyum, B.O., 1970. The protein quality of cassava leaves. Br. J. Nutr., 24: 761-768.
50. Iyayi, E.A. and O.O. Tewe, 1998. Serum total protein, urea and creatinine levels as indices of quality of cassava diet for pigs. Trop. Vet., 36: 59-67.
51. Kaneko, J.J., 1989. Clinical biochemistry of domestic animal. 4th Edn. Academic Press Limited, London, pp: 898.
52. Balogun, A.M., 1982. Biochemical and nutritional evaluation of some under-exploited forest and savannah crop seeds with emphasis on the anti-nutritional factor components. Ph.D. Thesis, University of Ibadan, Ibadan, Nigeria, pp: 150-155.

American-Eurasian J. Agric. & Environ. Sci., 2 (1): 99-102, 2007
ISSN 1818-6769

Evaluation of Crop Yield of African Yam Bean, Maize and Kenaf Under Intercropping Systems

O.N. Adeniyan, S.R. Akande, M.O. Balogun and J.O. Saka

Institute of Agricultural Research and Training,
Obafemi Awolowo University, P.M.B. 5029 Moor Plantation, Ibadan, Nigeria

Abstract: The effect of cropping systems on crop growth and yield of kenaf, maize and African yam bean (AYB) intercropped was investigated for two years (2003 and 2004) at the research farm of the Institute of Agricultural Research and Training (IAR and T) Ibadan. Significantly taller maize and kenaf plants were observed under maize/kenaf/AYB intercrop compared to other cropping systems. Highest seed yield of AYB was obtained in maize/kenaf/AYB and AYB/kenaf intercropping systems while the lowest seed yield was recorded from sole AYB. On the other hand, the highest grain yields of maize and kenaf were obtained when planted sole and in intercropped with AYB. The maize/kenaf/AYB intercropping system gave the highest value of land equivalent ratio (1.12) indicating that planting the three crops together gives higher productivity compared to sole cropping.

Key words: Yield % maize % kenaf % African yam bean % intercropping

INTRODUCTION

Intercropping is an age-old practice of cultivation used by the farmers of tropical and sub-tropical countries. Intercropping is believed to reduce risk and maximize farm revenue, in addition to the relatively high soil and labuor productivity [1]. Maize (*Zea may* L.) has been recognized as a common component in most intercropping systems. It was reported that about 75 percent of the area of maize in Nigeria is in association with other crops [2]. However, in Nigeria, the planting of African yam bean (*Sphenostylis stenocarpa Hoechst*) is usually in association with yam (*Dioscorea* sp.) in which the same stake serves as support for both crops [3]. It is also planted in association with maize and other crops [4]. Staking is as important in African yam bean grown as a sole crop as when grown in intercropped with other crops. Therefore, efforts have to be made to provide stakes for a better growth and yield.

Kenaf (*Hibiscus cannabinus* L.) is gradually gaining relevance in the intercropping system in some parts of the country because of its economic potential and role in the cottage fiber industry [1]. Intercropping fiber crop with legumes and cereals has been shown to give higher returns than sole cropping [5]. It has also been suggested that intercropping single rows of groundnut, soybean or black gram between paired or triple roselle rows spaced 15 cm apart would be commercially more viable than a sole roselle crop, particularly if the intercrops are sown 15 days after the roselle [6].

As a result of additional cost in providing staking materials in African yam bean cultivation as well as its growth that is very slow without staking, there is a need to device a means of intercropping the crop with other crops. However, little or no information exists on kenaf growing in association with cereal and or legume crops in rain forest agro ecology. The objective of this study, therefore, was to evaluate the effect of intercropping on crop growth and yield in Kenaf/Maize/African yam bean intercropping system under rainforest ecology.

MATERIALS AND METHODS

The study was conducted during the cropping seasons of 2003 and 2004 at the Research Farm of the Institute of Agricultural Research and Training, Moor Plantation, Ibadan (lat. 7° 22'N, long. 3° 50'E). This is in the lowland rainforest agro ecological zone of Southwest Nigeria. The average annual rainfall varies from 1000 to 1350 mm and has a bimodal distribution. The dominant soil of the experimental site is sandy loam of an alfisol. The experimental design was a Completely Randomized Block Design (CRBD) with three replications of 4×5 m

Corresponding Author: Dr. O.N. Adeniyan, Institute of Agricultural Research and Training, Obafemi Awolowo University, P.M.B. 5029 Moor Plantation, Ibadan, Nigeria

plot size. There were seven treatments; these are African yam bean (AYB)/Maize/Kenaf, AYB/Kenaf, AYB/Maize, Maize/Kenaf, sole maize, sole kenaf and sole AYB. Kenaf and maize were planted at the same time, while AYB was sown four weeks after. About four seeds of Cuba 108 (kenaf variety) and two seeds of DMR-ESR-Yellow maize variety were sown per hole in their respective rows and later thinned to two and one plant(s) per stand of the respective crop. The inter and intra row spacing for kenaf was 1×0.5 m (40,000 plants ha^{-1}), while maize was 1×1 m (10,000 plants ha^{-1}). The sole kenaf and maize seeds were sown at 0.5×0.2 m (100,000 plants ha^{-1}) and 0.75×0.25 m (53,320 plants ha^{-1}), respectively. Seeds of African yam bean were sown in row between rows of kenaf and maize plants at 0.5 m inter-rows and 0.8 m intra-row spacing (25,000 plants ha^{-1}). At emergence, the seedlings were thinned to one plant per stand. The plants were staked using bamboo poles where necessary with two plants per stake. Pest incidence was low and no insecticide was applied.

A basal application of 120 kg ha^{-1}. NPK (15-15-15) compound fertilizer was applied to maize and kenaf plants. At anthesis, a second dose of fertilizer was applied to maize in form of 40 kg N ha^{-1} using urea. Agronomic parameters taken were plant height at 12 weeks after sowing (WAS) and seed yield for kenaf. Plant height at 12 weeks after sowing (WAS) and grain yield were recorded for maize. While only seed yield was recorded for African yam bean. Data were analyzed using Genstat statistical package and Land Equivalent Ratio (LER) was estimated to test the productivity of the mixture using the following equation according to Fisher [7].

$$LER = Ya + Yb + Yc = Ya/Xa - Yb/Xb - Yc/Xc$$

Where; Ya + Yb +Yc is the total plot yield per unit land area.
Ya, Yb and Yc are the component yields for the three crops.

Xa and Xb are the yields per unit land area where a, b and c are grown under those conditions with which comparisons are to be made.

RESULTS

Maize: Cropping systems had significant effect on average plant height at 12 weeks after sowing (Table 1). The significant reduction in plant height at 12 weeks after sowing was observed in sole maize compared with intercrops. Maize/kenaf/AYB-intercropping system gave the highest average value (2.60 cm) at 12 weeks after sowing. It was observed that maize plant heights in maize/kenaf and maize/AYB intercropping systems were not significantly different from each other. The average grain yields obtained for sole maize, maize/AYB and maize/kenaf intercrops were significantly higher than that obtained in the maize/kenaf/AYB intercrop. The results indicated that, intercropping of the three component crops gave the lowest average value (1.48 t ha^{-1}). The effect of cropping systems on maize grain yield revealed that the sole maize>maize/AYB intercrop>maize/kenaf intercrop>maize/kenaf/AYB intercrop.

Table 1: Average maize plant height and grain yield in kenaf/maize/AYB intercrop

Treatments	Plant height 12 WAS (cm)	Grain yield (t ha^{-1})
Maize/Kenaf/African yam bean	2.60a	1.48c
Maize/Kenaf	2.40b	1.78b
Maize/African yam bean	2.38b	2.47a
Sole maize	2.09c	2.73a

Means with different letters within the same column are significantly different ($p<0.05$)

Table 2: Average kenaf plant height and seed yield in kenaf/maize/AYB intercrop

Treatments	Plant height 12 WAS (cm)	Seed yield (t ha^{-1})
Maize/Kenaf/African yam bean	2.86a	0.71c
Maize/Kenaf	2.33b	0.89b
Kenaf/African yam bean	2.66a	0.99a
Sole Kenaf	2.15c	1.02a

Means with different letters within the same column are significantly different ($p<0.05$)

Kenaf: Cropping systems significantly affected average plant height at 12 weeks after sowing and seed yield (Table 2). Tallest plants were observed under maize/ /kenaf/AYB intercropped, while the shortest plants were observed under kenaf sole cropping. The highest kenaf seed yield (1.02 t ha^{-1}) was recorded under kenaf sole cropping, although, this was not significantly different from that of kenaf/AYB intercropped.

African yam bean: Cropping systems significantly affected the seed yield of African yam bean. Highest seed yields were observed in AYB/maize/kenaf and AYB/kenaf intercropping systems compared to AYB/maize intercropped. Sole AYB had the lowest seed yield (Table 3).

Table 3: Average African yam bean seed yield in kenaf/maize/AYB intercrop

Treatments	Seed yield (t haG¹)
Maize/Kenaf/African yam bean	0.86a
Maize/African yam bean	0.64b
Kenaf/African yam bean	0.88a
Sole African yam bean	0.49c

Means with different letters within the same column are significantly different ($p<0.05$)

Table 4: Effect of kenaf-maize-African yambean cropping systems on the Land Equivalent Ratio (LER) in cropping seasons at Ibadan

Treatments	Land equivalent ratio
AYB/Maize/Kenaf	1.12
AYB/Maize/Kenaf	1.06
AYB/Kenaf	1.03

Land productivity: The resultant average Land Equivalent Ratio (LER) values for both years showed high yield advantages due to intercropping systems (Table 4). The LER value that was greater than 1.0, therefore, gave higher production efficiency. The average LER value (1.12) from the maize/kenaf/AYB intercropping system was the highest.

DISCUSSION

The highest maize plant height observed under maize/kenaf/AYB intercropped in this study could be due to the fact that when maize and kenaf were planted at the same time, the maize grew faster and stayed above the kenaf at the early stage. This is because in intercropping system, competition for light by component crops favors component crop with its leaf area higher in the canopy. Therefore, maize in intercropped with kenaf and AYB was at advantage because of its rapid growth rate due to competition for light when compared with maize sole cropping. It was reported that plant height and internodes length increased with increasing plant population because of competition for light [8]. The yield reduction in maize and kenaf obtained with intercropping of maize/kenaf/AYB and maize/kenaf compared with sole cropping of both crops were essentially due to competition from the component crops. Yield reductions involving one or all components in intercropping have been reported by other workers [8, 9]. They attributed such depressant effects to inter-specific competition for nutrients, moisture and/or space. On the other hand, AYB seed yield was significantly higher in maize/kenaf/AYB and kenaf/AYB intercropping systems compared to sole AYB and maize/AYB intercropped. The yield increased might probably be due to the advantageous effects of kenaf to serve as life stakes for the AYB. Intercropping significantly increased seed yield in AYB. However, due to the ability of kenaf to stay for longer period on the field than maize it appeared better in providing support for AYB. Apparently, the sole AYB faced stiffer problem of unavailability of stronger and stable staking materials throughout its physiological growth. The stakes provided could become weak due to the nature of the staking materials, termite infestation and rainstorm. Consequently, yield of AYB was increased with the provision of life stakes like kenaf for its efficient growth throughout its developmental period. The total Land Equivalent Ratios (LERs) obtained with intercropping which were all above 1.0 indicate that higher productivity per unit area was achieved by growing the component crops together than by growing them separately. The relatively high LER values obtained for maize/kenaf/AYB and kenaf/AYB intercrops compared to sole AYB and maize/AYB intercropped could be attributed to the yield benefits in AYB arising form ability of AYB to get life stakes for efficient growth and seed production.

CONCLUSIONS

Consequently, yield advantages were gained by growing maize, kenaf and Africa yam bean together. Due to the additional efforts in providing stake materials for efficient growth and development of AYB, substitutes could be successfully provided when farmers grow AYB with kenaf and/or maize. This will substantially improve growth and yield of the crop and the total yield obtained from other components will apparently compensate for the growing together of the three component crops.

ACKNOWLEDGEMENT

Mr. B. Idowu, Mr. S.O. Olabode and Miss. R.B. Olowoyo are highly acknowledged for their technical assistance.

REFRENCES

1. Kuchinda, N.C. and J.O. Ogunwole, 2000. Effects of dates and row arrangement on crop growth and yield in kenaf/maize mixture in the northern guinea savanna of Nigeria. J. Sustainable Agric. Environ., 2: 251-256.

2. Okigbo, B.N. and D.J. Greenland, 1976. Intercropping systems in tropical Africa. In: Papendick, R.I., A. Sanchez and G.B. Triplet (Eds.). Multiple Cropping. ASA Special Publication. No. 27. Madison WI USA, pp: 63-101.
3. Togun, A.O. and J.K. Egunjobi, 1997. Reproductive development and seed yield in African yam bean. Nig. J. Sci., 2: 29-35.
4. Saka, J.O., S.R. Ajibade, O.N. Adeniyan, R.B. Olowoyo and B.A. Ogunbodede, 2004. Survey of underutilized grain legume production systems in the Southwest agricultural zone of Nigeria. J. Agric. & Food Info., 6: 93-108.
5. Roquib, A., A.L. Kundu and B.N. Chartterjee, 1973. Possibility of growing soybean in association with other crops. Ind. J. Agric. Sci., 43: 792-794.
6. Roy, A.R., B.C. Casmal and A.K. Bhattacharjee, 1990. Effect of intercropping oil seeds and pulses in Roselle (Hibiscus *sabdariffa*). Experimental Agric., 26: 407-411.
7. Fisher, N.M., 1977. Studies in mixed cropping II. Population pressures in maize-bean mixture. Experimental Agriculture 13: 185-191.
8. Enyi, B.A.C., 1973. Growth rate of three cassava varieties under varying population densities. J. Agric., 81: 15-28.
9. Okpara, D.A. and C.P.E. Omaliko, 1995. Productivity of yambean (*Sphenostylis stenocarpa*)/yam (*Dioscorea* sp.) intercropping. Indian J. Agric. Sci., 65: 880-882.

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. Selegean, 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