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Extraction and Evaluation of Chitosan from Crab Exoskeleton as a Seed Fungicide and Plant Growth Enhancer

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Abstract: Proper disposal of seafood wastes is a continuous problem along the Eastern shores of the United States. Blue crab and scallop processing plants continuously dumped their residues into landfills, creating management and environmental concerns associated with ground and drinking water pollution. Additionally, build up of seafood waste generates an unpleasant odor and becomes an eye-sore to both tourists and local communities. Consequently, the present study was designed to evaluate alternate uses for seafood wastes that are economically feasible. The goal of this study was to isolate chitosan from crab exoskeletons and evaluate its potential as a fungicide against seed infection and a plant growth enhancer. Chitosan was obtained by first removing the shell-meat and recovering the calcium carbonate and proteins. Crab exoskeleton samples were demineralized with either 0.5% or 1.0% HCl or 5% or 10% CH_3COOH. A growth enhancement study was conducted with black-eyed peas exposed to the different chitosan treatments. A similar anti-fungal experiment with peanut seeds infested with *Penicillium* was conducted as well. Data on plants height, stem diameter and leaf counts were recorded biweekly for 4 months and analyzed using PROC ANOVA and PROC General Linear Model Statistical Systems. These measurements suggested that seeds pre-treated with the chitosan extracted with 0.5%HCl had the best overall growth and both chitosan (0.5% HCl) and Captan were the most effective in eliminating fungus from peanuts. This research presents plausible possibilities in which seafood waste can be utilized for agricultural purposes.

Key words: Seafood % crab wastes % chitosan % fungicide % plant-growth enhancer

INTRODUCTION

There are many products that utilize organic compounds from seafood waste. Chitin, a natural polymer found in crustaceans is currently being used in numerous medical applications such as bandages to prevent continuous bleeding or as a wound dressing, or to assist in controlling blood cholesterol [1-3]. It is most commonly used as a commercial dietary supplement because of its fat absorbing capabilities [1, 4]. Additional studies conducted show that chitin extracted from crab exoskeleton and its derivatives can be useful in environmental science, specifically for treating waste water and in agriculture as a plant fertilizer, plant growth enhancer and fungicide [1, 5-7]. The fact that discarded seafood can be utilized in a broad spectrum of health, medical and environmental fields, leads to a better solution for proper disposal of seafood wastes and prevention of excessive build up of seafood waste along the US East coasts.

Crabs are an important seafood product of the United States. The cumulative amount of domestic landings for crabs in both 2003 and 2004, were more than 290,000 metric tons [8]. In addition, more than 70 percent of seafood including crabs is considered processed waste material [9]. In Florida, disposal of seafood wastes is a

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continuous environmental concern. Residues from seafood processing plants dumped into landfills have created management and environmental problems related to water pollution [10, 11]. In fact, seafood waste disposal accounts for about 25% of the annual operating budgets of some Florida landfills [12, 13]. Disposal of residues in place other than landfills has produced economic strains for many processing plants, particularly those companies along the eastern shore of the United States (e.g. Florida, Georgia, North and South Carolina shores respectively) that may have already been struggling financially. In addition, build up of wastes tends to attract pests (e.g., flies and mosquitoes), pathogens, encourage bacterial growth, produce offensive odor and create an eyesore to tourists and local communities. The Florida Sea Grant College Program therefore developed a project to help eliminate seafood wastes. To alleviate the stench of the discarded seafood wastes and the burden of already stressed landfills with large amounts of seafood wastes, crab's scraps are mixed with sawdust and bark from trees to produce compost [12, 14, 15]. Other programs and studies have also conducted similar projects using crab-discarded materials and sawdust for various treatments [16-19]. The compost produced can be bagged and bulked in quantity for further use. Granted, discarded waste is utilized as compost, the bulk of seafood waste and its improper disposal is still a problem. This project was therefore designed to evaluate the chemical and physical properties of chitosan isolated from seafood wastes to be used in agriculture and environmental science. This research presented alternative methods of using processed seafood wastes to the scientific community and to government agencies. It also created an awareness of the value and use of organic compounds present in seafood waste. The overall goal of this research was to enhance the utilization of crab waste and help minimize environmental pollution associated with it. The specific objectives for this research were to extract chitosan from crab exoskeletons and 1) evaluate its growth enhancing properties for plants, 2) evaluate its anti-fungal properties for seed storage and preservation.

MATERIALS AND METHODS

Samples preparation: The crabs were obtained from Spears Seafood Market on Lake Bradford, in Tallahassee, Florida. The crab exoskeletons collected were placed in Ziploc bags and refrigerated overnight. Moisture content was determined on the crab waste by first crushing exoskeletons into smaller pieces using a meat tenderizer. Approximately 10 grams of crushed crab's exoskeletons wet samples were placed on foil paper and measured using a Mettle balance. There were five measurements made of the wet crushed crab exoskeletons samples. The samples were then labeled and oven-dried for 4 consecutive days at 65°C until constant weight. The dry weight of the samples were then determined and the moisture content measured based on the differences between the wet and the dry weight. The average moisture content of the crab exoskeletons was 12.96%.

Extraction of chitin and chitosan: The chitin and chitosan sequence involved the crushing and washing of the discarded exoskeletons as described by Kim [6] and by the Sonat Corporation [20]. The crabs' exoskeletons were placed in 250 ml beakers and treated in boiling sodium hydroxide (2% and 4% v/v) for one hour in order to dissolve the proteins and sugars thus isolating the crude chitin. Since there was little knowledge about what to expect from the 2% and 4% sodium hydroxide (NaOH) concentrations, the criteria established to assess the best results between the two concentrations were simply looking for any visible physical change such as color and/or texture. Based on the fact that both sodium hydroxide concentrations yielded no visual physical change in the crab exoskeleton, the 4% NaOH was selected for use in the chitin preparation, which is the concentration used by the scientists at the Sonat Corporation [20]. After the samples were boiled in the sodium hydroxide, the beakers containing the crab shell samples were removed from the hot plate, placed in the hood and allowed to cool for 30 minutes at room temperature. The exoskeletons were then further crushed to pieces of 0.5-5.0 mm using a Hamilton Beach, 7-speed blender.

Demineralization: The grounded exoskeletons were divided into 4 sub-crab samples weighing approximately 25 g each. Each sub-sample was demineralized with 100 ml of HCl using concentrations 0.5% or 1.0% and the remaining two samples with 5% or 10% acetic acid (CH_3COOH) concentrations. The samples were allowed to soak for 24 h to remove the minerals (mainly calcium carbonate). The demineralized crab shell samples were then treated for one hour with 50 ml of a 2% NaOH solution to decompose the albumen into water soluble amino-acids. The remaining chitin was washed with deionized water, which was then drained off. The chitin was further converted into chitosan by the process of deacetylation.

Deacetylation: The deacetylation process was carried out by adding 100 ml of 50% NaOH to each sample and then boiled at 100°C for 2 h on a hot plate. The samples were then placed under the hood and cooled for 30 min at room temperature. Afterwards the samples were washed continuously with the 50% NaOH and filtered in order to retain the solid matter, which is the chitosan. The prepared chitosan was then placed in 250 ml beakers and labeled according to the treatment used. The samples were then left uncovered and oven dried at 120°C for 24 h. The chitosan was then in a creamy-white form. The moisture percentage of the crab shell samples was then evaluated.

Growth enhancement: Black-eyed peas were selected for this study because it germinates quickly, known to grow well in sandy soil and is a common crop in Florida. They can withstand considerable drought and a moderate amount of shade. Black-eyed peas in Florida reach a canopy height around 20-24 inches. The germination of the seed is rapid at soil temperatures above 18°C. Black-eyed peas have a competitive niche in soils that are sandy [21].

The chitosan powder treatments (Table 1) were applied to black-eyed peas. The black-eyed peas that were treated in this experiment were purchased from Gramling's Incorporated on South Adams Street, Tallahassee, Florida. The chitosan treatments used in this experiment are listed in Table 1. There was also an untreated control for the growth enhancement experiment. The following procedure was used to pre-treat the seeds. Each chitosan treatment was used to treat 5 black-eyed peas in 4 replicates at 3 different time periods: 30 min, 60 min and 120 min. The reason for evaluating time intervals for pre-treatment was to establish whether time exposure to the chitosan powder would have any effect on the biological response of the seeds. The seeds were pre-treated by first shaking the seeds and the powder vigorously for one minute, every 10-minute interval, in a flat glass pan sealed with an air tight rubber cover. This was to ensure even distribution of the chitosan powder on the seeds. The treated seeds and the untreated group (control) were then sown in small plastic trays (3 x 5") containing topsoil. The chitosan treatments were used to treat 20 black-eyed peas and 4 were sown per tray. The trays were then placed outdoors in an enclosed area. Each tray was labeled according to the treatment used on the seeds. Germination and seedling growth was evaluated for 14 days for each treatment.

Chitosan fungicidal action: Chitosan powder treatments and the commercial fungicide Captan were used to treat the peanuts infected with the fungus *Penicillium*. The purpose of this experiment was to test the action of chitosan as a fungicide by eliminating the fungus, over a six-week period compared to a commonly used commercial product. Naturally infected peanuts with the fungus *Penicillium* were provided by Dr. Onokpise, Department of Forestry and Agronomy, College of Engineering Sciences, Technology and Agriculture, Florida A&M University, Tallahassee, Florida. The procedure for conducting this experiment was as follow. The chitosan treatments were used to treat the peanuts. The control used was Captan, a white-powdered commercial fungicide. There was also a non-fungicide treatment to monitor the growth rate of the fungus. A total of 15 peanuts with 5 peanuts per sterilized Petri dish were used for each of the chitosan treatments and control. The fungus equally covered the circumferences of each peanut under investigation. The infected peanuts used, were stored in small tightly sealed plastic containers in a growth chamber (LAB LINE, Model 845) at a temperature of 22°C and relative humidity of 22% until needed for the experiment.

To treat the infected peanuts, they were held with forceps to minimize disturbance of the fungus and then the chitosan powder and Captan were lightly brushed on the seed coat. The treated peanuts were then placed in sterilized petri dishes and maintained in the growth chamber at a 16/8 hour photoperiod for monitoring. The peanuts were evaluated by microscopic observations biweekly for six weeks in order to investigate the rate and success of the chitosan treatments between each other and the Captan in eliminating the fungus. Results were based on inferences made visually by noting the number of peanuts from which the fungus was eliminated when treated with the chitosan treatments and the commercial fungicide Captan. The average and standard error for the amount of peanuts from which the fungus was eliminated for each treatment at the end of the six week study were recorded. This experiment was repeated for another six weeks using 5 infected peanuts per treatment in 3 replicates and data were recorded biweekly. Each time the measurements were recorded, if there was no fungal detection on any of the peanuts it was discarded and recorded.

Field study: The field study was conducted from August to December. For the field studies, 5 black-eyed peas in each of 3 replicates were treated using the chitosan treatments at the best time period (Table 1) established for

germination and seedling growth in the preliminary studies. This was done by first shaking the seeds and the powder vigorously for 30 minutes at 10 minute intervals for 1 min. The powder and the seeds were as described above. The seeds were then stored in Ziploc bags and the treatments were labeled according to the acid concentrations that were used for demineralization for 24 hours at room temperature.

Before the seeds were sown, the soil pH, nitrogen and moisture content were determined in the Environmental Sciences Institute at Florida A&M University, Tallahassee, Florida. The College of Agriculture and Environmental Sciences Laboratory in Athens, Georgia analyzed the nitrogen levels of the soil samples. Field plots were 60" x 60" comprised into rows, each labeled according to chitosan treatments. The treated seeds and the control were then sown in a Randomized Block Design in 3 replicates throughout the experimental area. The seeds were sown 3 feet apart from one another to allow sufficient growth space.

To evaluate chitosan effects on the plant morphogenesis, i.e. average height, stem diameter and leaf number per plant were recorded. The height of the plant was measured as the distance start from the base of the stem near the soil and extended to the apical bud of the plant. The stem base diameter was measured using a 6 inch dial caliper. All these measurements were in centimeters.

The leaves produced for each plant per treatment were counted and then their average numbers per each treatment calculated. Data collection started at the beginning of the second month, in order to allow the plants to fully establish. Measurements were then recorded biweekly within the second month until the end of the fourth month. The measurements were evaluated and analyzed for variations between each of the chitosan treatments and the untreated control. To ensure successful growth of the black-eyed pea plants, the weeds were uprooted biweekly from around the area in which the plant was established, reducing competition from soil nutrients.

Rainfall during the four month study period was recorded biweekly with a Herd Health Pluviometer reading. The average temperatures for each month throughout the four-month study period were recorded. The data for the rainfall during that period was recorded by the employees at the Florida A&M University Research Center in Quincy, Florida. Other observations were noted throughout the experiment such as whether pests attacked the plants causing deterioration or any other biotic and abiotic stress.

At the end of the experiment, soil samples were collected and analyzed for pH, nitrogen and moisture contents. The reason for analyzing the soil's conditions at the end of the experiment was to determine whether chitosan caused any changes in the soil conditions that may have some effect on the growth of the plants. The soil samples were collected approximately 7 inches deep using a soil samples tool. There were 3 soil samples as replicates taken from each treatments area and the untreated control, from points exactly where the plants were grown. The soils samples were then air dried for 3 days and placed in sealed glass jars and labeled according to the area they were collected. For further analysis, the seeds treated with chitosan were then placed in Ziploc bags, labeled according to the area from which they were collected and transported on ice to prevent chemical alterations.

The moisture content of the various soil samples was analyzed and recorded. The pH of the soil was analyzed by first dissolving 30 g of the 3 soil samples form each treatment area, in 100 ml of deionized water. The pH meter then was calibrated and the pH of the soil for each treatment area was then analyzed and recorded. These soils analyses were done in the Environmental Sciences Institute at Florida A&M University, Tallahassee, Florida.

Data analysis: For the growth enhancement and fungicide studies, the data was analyzed using the PROC ANOVA and PROC General Linear Model (GLM) Statistical Analysis Systems (SAS) with a 95% significance level. The differences between the averages were compared using Duncan Multiple Range Test (DMRT). For the growth enhancement experiment, the data were analyzed for the differences between the average percentage germination rates for seeds exposed to chitosan treatments at the various time periods. For the fungicide studies, the data was analyzed and the differences between averages and the standard deviation from which fungus were eliminated on the peanuts for each treatment was indicated. For the field studies, the data was analyzed for the differences between the average leaf count, stem diameter and height for the treatments used and the untreated control. The results that have the same lower case letters indicate that there was no significant difference between them (Table 2).

RESULTS

Samples preparation: The results of the samples preparation are presented in Table 1. The samples

Table 1: Samples preparation and the different treatments used in the study

Crab exoskeleton samples (g)	Wet weight of crab shells (g)	Dry weight of crab shells (g)	Moisture content of crab shells (g)	Acid concentrations used for demineralization	Chitosan treatments
25.2	45.50	26.27	42.10	0.5% HCl	$Chitosan_{0.5\%HCl}$
30.9	66.90	39.10	45.50	1.0% HCl	$Chitosan_{1.0\%HCl}$
25.7	34.90	19.30	44.35	5% CH_3COOH	$Chitosan_{5\%CH3COOH}$
25.1	39.40	24.20	34.90	10%CH_3COOH	$Chitosan_{10\%CH3COOH}$

Table 2: Effects of chitosan treatments on growth of black-eyed peas, fungus-infected peanuts and soil nutrition

	Seed germination %							Plant morphogenesis		
	exposure time (min)			Fungal	Soil					
Treatments	30*	60	120	elimination from Peanuts %	moisture content %	pH level	Nitrogen level	Mean leaf count	Mean plant height (cm)	Mean stem diameter (cm)
0.5% HCl	90a	60b	80a	**3.7a	14.3	6.1a	0.0222%	***2.88ab	8.81a	0.33a
1% HCl	80a	60b	60b	2.8b	14.9	6.3a	0.0228%	3.29a	7.96bc	0.28b
5% CH_3COOH	75a	55b	65b	2.7b	14.0	6.1a	0.0230%	3.04ab	8.28bc	0.26b
10% CH_3COOH	85a	55b	40b	2.6b	15.1	6.2a	0.0220%	2.65b	7.25c	0.25b
Control				3.3a	10.8	6.2a	0.0237%	1.80c	5.56d	0.18c
					16.1 (IMC)	6.4a (SP)	0.242% (INL)			

The values listed in the table represent average counts of the replicates taken over the respective growth for the plants or exposure period for the fungicide

*Length of time, the seeds were exposed to the chitosan. **Average number of peanuts from which the fungus was eliminated after exposure to chitosan. ***Within a column, data with the same letter are not significantly different at the 5% level ($P<0.05$)

IMC (Initial Moisture Content of the soil), SP (Starting pH of the soil at the beginning of the experiment), INL (Initial Nitrogen Level of the soil at the beginning of the study)

preparation included extraction of chitin and chitosan, demineralization and deacetylation of crab exoskeletons. For each treatment used (Table 1), the amount of crab exoskeleton collected varied from 25 to 30 g, while the wet weight of crab ranged from 34 to 67 g. The samples preparation included the dry weight and the moisture content of the crab samples as well, which ranged from 19 to 39 g and 34 to 45 g, respectively.

Growth enhancement: The results for the laboratory studies enhancement experiment are shown in Table 2. The data represent the percentage seed germination after being treated for the various time periods. Results showed that the seeds exposed to chitosan for 30 min prior to planting achieved a better germination percentage when compared to those exposed to chitosan for 60 and 120 min respectively. Those exposed for 60 min showed less germination than those treated for 120 min, however there was no significant difference between these results. The seeds exposed for 30 min to chitosan showed higher germination rates (%) compare to the untreated control. Also, the seeds exposed to chitosan treatments were taller than the untreated control. Furthermore, the seeds treated with chitosan 0.5% HCl for 30 min, produced the greatest percentage of germination compared to the other treatments. However, the germination ratio (%) for plants exposed to chitosan 0.5% HCl was homogenous with the one of the untreated control.

Chitosan fungicidal action: The results of the laboratory studies for the fungicide experiment at the end of the 6-week period are shown in Table 2. The total number of peanuts that were exposed to each treatment was 15. Within the first two weeks, none of the treatments were effective in reducing or eliminating the fungus, because *Penicillium* was still present on all of the peanuts. At the end of the fourth week, chitosan 0.5% HCl, chitosan 1.0% HCl and chitosan 5% CH_3COOH treatments eliminated fungus from 53% of the peanuts. The chitosan 10% CH_3COOH treatment and control eliminated 47% of the fungus.

At the end of the six-week period, chitosan 0.5% HCl eliminated fungus from 73% of the peanuts; chitosan 1% HCl eliminated fungus from 60% of the peanuts; chitosan 5% CH_3COOH eliminated fungus from 57% of the peanuts and the control eliminated fungus from 67% of the

peanuts. From these studies, chitosan 0.5% HCl and the control showed the best response as anti-fungal agents under laboratory conditions. There was no significant difference ($P<0.05$) between the chitosan 0.5% and the control.

Field study: The results of the field studies shown in Table 2, presented average leaf count, height and stem diameter of the plants that were under investigation for the four month study. The results for average leaf count of plants whose seeds were treated with chitosan 1.0% HCl and chitosan 5% CH_3COOH treatments, revealed better leaf production. The average number of leaves when treated with chitosan 1% HCl and chitosan 5% CH_3COOH were 3.29 and 3.04 respectively. The average leaf count for the untreated control was 1.80 therefore were less than the plants whose seeds were treated with chitosan treatments. The average height of the plants treated with chitosan was greater than the untreated control. However the height of the plants treated with chitosan 0.5% HCl and chitosan 5% CH_3COOH was better than all other plants. The average height for chitosan 0.5% HCl and chitosan 5% CH_3COOH treatments were 8.81 and 8.28 cm respectively. The overall results for the untreated control in regard to the height of the plant were significantly lower than the experimental plants, therefore the height growth process was much slower for the controls.

For evaluations of the stem diameter, plants in which the seeds were treated with chitosan 0.5% HCl gave the best results indicating that the plants readily established themselves. The average stems diameter of the plants when treated with chitosan 0.5% HCl was 0.32 cm. For the untreated control, the average stem diameter was 0.18 cm. There were also some pest and predator activities during the study which might have affected the growth success of the plants. Pests such as beetles, caterpillars and butterflies attacked the plants deteriorating the quality of the leaves of the plants. Also deer trampled through the experimental area disturbing the growth process of some of the plants. However all plants were exposed to the same conditions at all times.

DISCUSSION

According to the results of the growth enhancement experiment conducted in the field, it appears that the chitosan treatments were successful in giving a better plant growth than the untreated control. Chitosan, which is chemically an amino sugar, ß-D-glucosamine, may have assisted in stimulating the synthesis of protective agents [7]. Chitosan and its oligosaccharides contributed to plant growth by acting as natural elicitors or Catalysts inducing pathogenesis related proteins such as chitinase enzymes for young seedlings. As indicated by Ohta *et al*., [7], chitinase enzyme is already present in the plant as a defense mechanism but with the assistance of chitosan the defense mechanism is stimulated earlier than the normal cycle of the seedlings growth therefore providing protection at an earlier stage.

In black-eyed peas, chitosan may have acted as a signal for cellulose response of the chitinase enzyme in the plants to initiate their defense mechanism against phytopathogenic infections. Once the defense mechanism of the plants was initiated by the chitosan and chitinase enzyme activities, the plants were able to grow more productively without undergoing any deterioration due to infection. Chitosan inducing the defense mechanism of the plants also provides protection against environmental stress such as drought and maintains stability of the plant. For instance, the stems of the chitosan treated plants were much thicker than the untreated control. As the temperature decreased from August to December the stems of the treated plants continued to thicken. The stems of the untreated control however were much thinner during the dry season, which was between November and December.

A previous study was done on the effect of chitosan treatments on plants during heat stress by Duke and Doehlet [22]. The results of their study were consistent with the idea that increased heat affected the productivity of plants. However with decrease in temperature, producing cold stress during the growth enhancement study, chitosan was still able to maintain healthy growth. This then presents the idea that if modifications are made to the production and application of chitosan, the plants should be able to grow productively under heat stress.

Since the nitrogen levels in the soil samples for areas where chitosan treatments on plants were used were lower than the soil samples of the control, this indicated that chitosan may have induced the absorption capacity of the treated plants (Table 2). Therefore nitrogen was readily taken up by chitosan treated plants contributing to their successful growth. Also chitosan alone contains approximately 6.8% of all other minerals present in the crab shells, therefore the additional nitrogen was utilized by the chitosan treated plants [23].

The ß-D-glucosamine, chitosan also contributed to the absorption of water present in the soil. The more moisture in the soil, the more ß-D-glucosamine becomes

present increasing the plants absorption ability. Chitosan also assisted in conserving water in the plants by closing the stomata and decreasing transpiration and maintaining production of plants [24]. As a result, during the dry season when rainfall was low, the chitosan treated plants were able to provide themselves with water to prevent wilting and continued to circulate the nutrients in the plant for successful growth. Therefore the numbers of leaf foliage for chitosan treated plants in this study was greater than the untreated control due to the ability of these plants to conserve more water. The untreated control during the dry season was not able to provide itself with additional water, so began to deteriorate as the season changed. At the end of the experiment the pH level of the soil for the various sample areas including the untreated control slightly decreased from the initial pH of the soil. The pH of the soil was between 6.0 and 6.5 (Table 2). The change in pH of the soil was not significant, therefore had no tremendous impact on the growth of the plants.

Though the chitosan treatments were more successful in growth compared to the untreated control, there were some variations in the results among the chitosan treated plants. For instance the plants treated with chitosan O.5%HCL gave the best overall result in the growth enhancement study because it had the best results for 2 of the 3 characteristics that were under investigation. The characteristics included achieving a greater height and stem diameter. The chitosan l.0% HCL treatment achieved a greater percentage for leaf production. Although plants treated with chitosan 5%CH_3COOH did not give the best overall results. It had a relatively good performance in terms of percentage for average leaf count and height of the plants.

The 0.5% HCL acid that is commonly used by scientists in the demineralization process [25] and used in this experiment for chitosan production, can therefore be substituted with a weak, organic and more environmentally friendly acid such as the acetic acid used in the demineralization process. Since chitosan 5% CH_3COOH produce significant good results in the growth enhancement study, it therefore has the potential to successfully be applied to other environmental and agricultural areas once further modifications are made to the methodology to enhance chitosan production. The results for chitosan 10%CH_3COOH treatment after evaluating the success of the plants average height, leaf count and stem diameter were significantly lower than the results of the other chitosan treatments. This indicated that a higher percentage of acetic acid used did not produce beneficial results. However the results for plants treated with chitosan 5%CH_3COOH were more successful than the results of the untreated control. The results for this study were compatible with the growth enhancement results done by Goosen [26].

As for the effects of chitosan and Captan treatments on fungal growth, chitosan 0.5%HCl treatment gave the best result in eliminating and inhibiting fungal growth from the peanuts for both the preliminary and final study. Chitin is a protein that is present in the fungal cell walls, assisting with cell structure. However chitosan hydrolyzes chitin in the cell walls and deteriorate the cell structural component, such as cell wall thickening and hyphal distortion [27]. Chitosan has good adherence capabilities. Once applied to the seed coat of the infected peanuts, it immediately adhered to the fungus and began its reaction. Chitosan acted as a catalyst stimulating chitinase enzymes, initiating the defense mechanism of the peanut. Once the defense mechanism was initiated, the fungal cell walls underwent some disturbances. The chitosan began to destroy the cell walls by causing leakage of amino acids and proteins which support the fungal cell walls causing cytological damage [5]. As a result the cell walls began to erode. Therefore chitosan 0.5% HCl treatment was consistent with its effectiveness for plant growth enhancement as well as a fungicide.

However, the commercial product captan was more effective than chitosan 1%HCl, chitosan 5%CH_3COOH and chitosan 10%CH_3COOH treatments in inhibiting and eliminating the fungal growth of *Penicillium.* Research conducted previous to this study, indicated that the commercial fungicide prochloraz was more effective in eliminating *Penicillium* than the chitosan treatments under investigation [28]. However the Chitosan 0.5%HCl treatment gave better results for fungal elimination than the commercial fungicide captan. Of course the prochloraz may be a more effective fungicide than captan, but its effectiveness compared to chitosan treatments in this study is yet to be investigated. Therefore further investigations can be conducted to evaluate fungal elimination and inhibitory activity of the chitosan 0.5%HCl treatments compared to other commercial fungicides that are being used today.

There was one difference in the results between the laboratory studies of the fungicide experiment and the final fungicide experiment. The number of peanuts in which the fungus was eliminated was greater for the laboratory studies than the final experiment. This is because the fungal growth on the peanuts for the laboratory studies was less abundant on the

circumference compared to the peanuts in the final experiment. As a result, the chitosan treatments and captan were able to eliminate the fungal growth more readily. However from simple microscopic observations, the fungus on the peanuts in the final fungicide experiment did undergo some reduction on the circumference of the peanuts that were treated compared to the initial abundance of fungus that was present. This indicated that given a longer time of exposure to treatments, eventually the fungal growth of the *Penicillium* would have been significantly reduced.

CONCLUSIONS

Improper disposal of seafood waste materials has become a serious environmental concern for coastal areas. This research introduces plausible possibilities in which seafood waste can be utilized for agricultural purposes. The use of natural waste products within the environment can not only eliminate build up but also reduce excessive use of chemicals in the environment that may result in terrestrial and aquatic pollution. From the results of the present research specifically, the growth enhancement experiment, the chitosan was successful in increasing the growth of plants and can be a potential fertilizer. Though its fertilizing capability was not compared to any organic and inorganic commercial fertilizer that are being used, it still presented the possibility of providing similar results when compared to commercial fertilizers. Chitosan when treated on the seeds, has the potential to inhibit microbial and pathogenic activity. Therefore the amount of commercial pesticides that are used and enter into our water sources through runoff may be reduced. Chitosan entering our water sources may not cause adverse effects on the environment, since it is a compound found in nature and only small amounts are used. Chitosan can then be evaluated as a pesticide. Other considerations when using chitosan should be evaluated under various abiotic conditions such as change in temperature, pH and salinity levels. We can increase or evaluate the effectiveness of chitosan on plant growth during changes in seasons, in various types of environments such as tropical or coastal inlands and whether these abiotic factors may also contribute to promoting chitinase enzymes increasing plant growth.

Since chitosan provides a protective layer against bacteria and fungi that may result in decay, it may be considered a good source for preservation of seeds and fruits during storage. The chitosan preservative ability then can be compared to commercial preservative that are being used today. The use of chitosan and other processed seafood waste material for agricultural purposes, instead of some commercial fertilizers and fungicides that are being used today, can prevent excess amounts of chemical buildup that may enter the environment resulting in pollution.

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Seed Germination and Early Root Growth of Three Barley Cultivars as Affected by Temperature and Water Stress

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Abstract: Effects of temperature (9, 15, 21, 27 and 33°C) and water stress (water potentials of 0, -0.3, -0.6 and -0.9 MPa) on germination and early rooting of three barley (*Hordeum vulgare* L.) cultivars were studied. High temperatures (27, 33°C) reduced germination rate and percentage, but these effects were more pronounced under high stress conditions. Main axis and total root lengths were highest at temperatures 21 and 27°C regardless of water stress level, while the highest number of seminal roots was obtained at temperatures between 15 and 27°C. Water stress caused significant reductions of both germination and rooting characters. The cultivar Rehani -3 had the highest germination percentage and rate among studied cultivars, while SLB-6 cultivar had generally the highest main axis and total root lengths regardless of temperature and water stress level, which may indicate that roots of this cultivar could elongate rapidly which ensuring better water supply under arid and semiarid conditions.

Key words: Germination % *Hordeum vulgare* % root elongation % arid region % water potential

INRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most widely grown crops in arid and semiarid regions of the world. It is grown mainly as feed grain or it can be grazed before heading in dry years. Seeding at the optimum date (usually in fall) or even earlier is important in barley production in Mediterranean arid regions. High evaporation rates due to high temperatures may occur during the common sowing time of barley, resulting in a rapid drying of surface soils after being wetted and causing a marked decrease in soil water potential [1, 2]. The seeds of barley show a delayed or reduced germination when the water potential of surrounding medium decreases [1, 3, 4]. Also the sensitivity to water stress differs greatly in relation to genetic and environmental factors [5-7].

The effect of unfavorable weather is probably more critical during germination and early seedling development stages than at any other stage of vegetative growth. Successful germination of the seeds under a wide range of environmental conditions (e.g., temperature and moisture) is important for early seedling establishment [8-11]. The rate and final percentage of germination of cereals are affected by moisture stress and temperature [1, 12]. The results of early studies on the effects of temperature on different plant species showed that there was a broad optimum temperature for the growth of seedlings [9, 10, 13]. However, seedling establishment may be hindered by a rapid drying of surface soils after being wetted [1, 14]. Therefore, plants grown in these environments should have the ability to develop a root system rapidly once seed germination has occurred, in order to ensure a continuous water supply for transpiration and growth. Sometimes, the plant's need for water can be met by a rapidly elongating main axis [9], while in other situations many long laterals are needed to exploit the water found in a thin layer of wetted surface soil.

Little information was found in the literature about the combined effects of temperature and water stress on germination and early root growth of different barley cultivars. The objectives of this study were to test the hypothesis that seed germination and early root growth of barley cultivars are affected by temperature and water stress and to determine if genetic variability exists

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between barley cultivars in response to these stress factors.

MATERIALS AND METHODS

Seed lots of the barley cultivars Rehani-3, SLB-6 and Rum were used in this study. Seeds of all cultivars had similar initial moisture contents of 8-10%. Thirty seeds from each cultivar were germinated on two layers of Whatman filter paper, in 9-cm petri dishes containing different potentials of osmotic solution created by adding polyethylene glycol 8000 (PEG) at 0, 16.0, 19.5 and 22.5% in solution (W/W) (equivalent to approximately 0, -0.3, -0.6 and -0.9 MPa, respectively). The petri dishes were placed in an incubator at the appropriate temperature (9, 15, 21, 27 or 33°C) in the dark. The treatments were placed in a factorial arrangement in a completely randomized design with four replications. Distilled water was added to each petri dish to replace the evaporated water.

Seeds were considered germinated when they exhibited radicle extension of >3 mm. Counts of germinated seeds were made daily during the course of the experiment to determine both final germination percentage and germination rate index. Final germination percentages were calculated from total number of seeds germinated divided by total number of seeds used. The germination rate index was determined by using the procedure described by Al-Karaki [1]: Germination rate index = $\Sigma G_n/D_n$

Where, G_n are germination percentages at different days (D_n) after initiation of germination.

The experiment was terminated by harvesting seedlings three days after no more germination occurred. After the Main Axis Root Length (MARL) was measured by a ruler and Seminal Root Number (SRN) was counted for each seedling, the seedlings were separated into shoots and roots. The total root root mass was weighed to determine Root Fresh Weight (RFW). Ttotal Root Length (TRL) were measured according to the procedure described by Al-Karaki [1].

Data were statistically analyzed using analyses of variance in the MSTATC PROGRAM (Michigan State Univ., East Lansing, MI). Probabilities of significance were used to indicate significance among treatments and interaction effects. LSD ($p<0.05$) was used to make comparisons among means.

RESULTS

The temperature (T) and water potential (WP) treatments and the T×WP interactions provided numerous significant responses for most of the studied plant traits (Table 1). Cultivars (C) had significant differences for all germination and rooting traits except for root fresh weight. Other significant interaction effects were T×C and WP×C for final germination percentage and rate index and T×WP×C for germination rate index.

In all cultivars, the final germination percentage was highest at 15°C and started to decrease as the temperature increased with the lowest at 33°C, regardless of water potential (Table 2). Final germination percentage at 9°C was significantly lower than at 15°C only for the cultivars Rum and Rehani-3. As water potential in the medium decreased (0 to-0.9 MPa), the final germination percentage was decreased in all cultivars. The genotypic differences in response to temperature and water potential for final germination were highly significant. The cultivar Rehani-3 had higher final germination percentage than the other two cultivars regardless of temperature or water potential (Table 2). However, the cultivar Rum generally had the lowest final germination percentage regardless of temperature or water potential.

The germination rate index decreased (more time needed for germination) as water potential of medium decreased (more stress) in all cultivars (Table 3). Highest germination rate of the seeds of all cultivars occurred at 15 and 21°C and decreased thereafter with increasing temperature regardless of water potential. The cultivar Rehani-3 had higher germination rate than the other two cultivars regardless of temperature or water potential. The

Table 1: Probabilities of significance for different traits of barley as affected by Temperature (T), Water Potential (WP) and Cultivar (C)

Trait	T	WP	C	T×WP	T×C	WP×C	T×WP×C
Final germination	**	**	**	**	**	**	ns
Germination rate	**	**	**	**	**	*	*
Main axis root length	**	**	*	**	ns	ns	ns
Total root length	**	**	*	**	ns	ns	ns
Seminal root number	**	**	*	ns	ns	ns	ns
Root fresh weight	**	**	ns	**	ns	ns	ns

** and * are significant at $p<0.01$ and $p<0.05$, respectively. ns: not significant

Table 2: Effects of temperature and Water Potential (WP) on final germination (%) of three barley cultivars

		Temperature (°C)				
WP (-MPa)	Cultivar	9	15	21	27	33
0	Rum	80 b-g¶	87 a-d	70 d-j	52 k-o	30 r-u
	Rehani-3	91 ab	98 a	91 ab	67 f-k	62 h-m
	SLB-6	90 a-c	91 ab	91 ab	50 l-p	35 p-s
0.3	Rum	74 d-I	84 a-e	54 j-n	29 r-v	27 r-v
	Rehani-3	91 ab	98 a	84 a-e	59 i-m	58 i-m
	SLB-6	84 a-e	89 a-c	85 a-e	49 m-q	24 r-v
0.6	Rum	67 f-k	83 a-f	54 j-n	28 r-v	20 t-v
	Rehani-3	89 a-c	98 a	81 b-j	57 j-m	40 n-r
	SLB-6	82 a-g	83 a-f	69 e-d	33 q-s	23 s-v
0.9	Rum	66 g-l	76 c-h	36 o-s	13 v	14 u-v
	Rehani-3	78 b-h	87 a-d	78 b-h	34 p-s	32 r-t
	SLB-6	78 b-h	80 b-j	59 i-m	20 s-v	16 t-v

Values followed by the same letter(s) are not significantly different (p = 0.05) according to LSD

Table 3: Effects of temperature and Water Potential (WP) on germination rate index of three barley cultivars

		Temperature (°C)				
WP (-MPa)	Cultivar	9	15	21	27	33
0	Rum	16.2 n-w¶	24.6 f-m	22.5 g-p	19.2 k-r	8.2 w-y
	Rehani-3	22.7 f-p	43.1 ab	48.5 a	36.0 b-e	33.5 c-e
	SLB-6	19.6 i-r	30.9 d-f	33.9 c-e	17.8 l-t	9.9 t-y
0.3	Rum	15.5 o-w	25.7 e-l	24.1 f-n	14.5 p-w	11.7 r-x
	Rehani-3	21.3 h-q	29.8 d-j	40.7 a-c	28.9 d-h	26.7 e-k
	SLB-6	17.2 m-v	27.9 d-i	39.4 bc	27.6 e-j	13.3 q-x
0.6	Rum	15.1 o-w	23.0 f-o	24.6 f-m	5.7 xy	9.0 u-y
	Rehani-3	19.5 j-r	28.4 d-h	42.7 ab	22.4 g-p	17.3 m-u
	SLB-6	18.3 l-s	27.6 e-j	25.0 f-m	13.1 q-x	8.9 v-y
0.9	Rum	13.1 q-x	22.7 f-p	10.1 s-y	2.5 y	2.0 y
	Rehani-3	18.1 l-t	23.5 f-o	30.4 d-j	13.2 q-x	9.8 t-y
	SLB-6	19.1 k-r	24.1 f-n	22.4 g-p	5.9 xy	2.7 y

Values followed by the same letter are not significantly different (p = 0.05) according to LSD

Table 4: Effects of temperature and Water Potential (WP) on main axis root length (cm/seedling) of three barley cultivars

		Temperature (°C)				
WP (-MPa)	Cultivar	9	15	21	27	33
0	Rum	1.9 q-t¶	5.8 f-l	9.2 a-d	10.2 ab	6.9 d-I
	Rehani-3	1.8 r-t	5.8 f-l	7.2 c-h	9.9 ab	6.4 e-j
	SLB-6	2.5 o-t	6.4 e-j	10.0 ab	10.5 a	7.3 c-h
0.3	Rum	1.9 q-t	6.0 e-k	5.0 h-n	7.4 c-g	5.1 g-n
	Rehani-3	1.8 r-t	4.3 j-p	7.0 c-i	8.0 b-f	7.0 c-i
	SLB-6	2.1 p-t	5.8 f-l	8.2 a-e	9.3 a-c	6.1 e-k
0.6	Rum	1.3 st	4.5 j-o	4.2 j-q	7.1 c-h	3.8 k-r
	Rehani-3	1.8 r-t	4.7 i-o	6.0 e-k	6.0 e-k	5.2 g-n
	SLB-6	2.1 p-t	6.0 e-k	7.2 c-h	7.2 c-h	5.1 g-n
0.9	Rum	1.4 st	3.2 m-t	3.1 n-t	4.2 j-q	3.6 l-s
	Rehani-3	1.2 t	3.8 k-r	3.9 k-r	6.5 e-j	4.3 j-p
	SLB-6	2.1 p-t	5.5 g-m	4.7 i-o	4.7 i-o	5.0 h-n

Values followed by the same letter(s) are not significantly different (p = 0.05) according to LSD

Table 5: Effects of temperature and Water Potential (WP) on total root length (cm/seedling) of three barley cultivars

		Temperature (°C)				
WP (-MPa)	Cultivar	9	15	21	27	33
0	Rum	6.5 r-y¶	18.0 g-m	27.9 a-c	30.0 ab	19.0 f-l
	Rehani-3	4.6 w-y	16.6 n-q	25.5 a-f	24.6 a-g	20.2 d-j
	SLB-6	7.6 r-y	19.4 f-k	26.3 a-e	31.3 a	26.7 a-d
0.3	Rum	4.8 w-y	16.9 h-p	16.2 h-q	19.7 e-k	15.6 h-q
	Rehani-3	4.7 w-y	12.1 m-u	18.8 f-m	21.3 c-h	17.5 h-n
	SLB-6	5.5 u-y	16.7 h-q	24.4 b-g	29.5 ab	19.3 f-k
0.6	Rum	5.1 w-y	14.4 i-r	15.4 h-q	16.7 h-q	12.4 l-t
	Rehani-3	3.7 wy	11.1 n-w	19.5 e-k	16.1 h-q	15.2 h-q
	SLB-6	5.6 t-y	14.8 h-q	20.7 d-i	19.9 d-k	17.3 h-o
0.9	Rum	3.5 y	10.1 p-y	10.5 o-x	13.3 k-s	13.8 j-r
	Rehani-3	3.7 wy	10.0 q-y	10.6 o-w	15.6 h-q	13.4 j-r
	SLB-6	5.0 w-y	15.7 h-q	13.9 i-r	15.7 h-q	16.6 h-q

Values followed by the same letter(s) are not significantly different (p = 0.05) according to LSD

Table 6: Effects of temperature and water potential (WP) on seminal root number of three barley cultivars

		Temperature (°C)				
WP (-MPa)	Cultivar	9	15	21	27	33
0	Rum	2.1 g-l¶	3.4 a-e	3.8 a	3.6 a-c	3.2 a-g
	Rehani-3	1.8 j-l	3.1 a-h	3.2 a-g	2.5 c-l	2.3 e-l
	SLB-6	2.4 d-l	3.5 a-d	3.8 a	3.7 ab	3.2 a-g
0.3	Rum	2.0 h-l	3.1 a-h	2.9 a-j	2.4 d-l	2.6 b-k
	Rehani-3	1.9 i-l	2.8 a-j	2.8 a-j	2.4 d-l	2.3 e-l
	SLB-6	2.2 f-l	3.5 a-d	3.4 a-e	3.5 a-d	3.0 a-i
0.6	Rum	1.8 j-l	3.3 a-f	2.6 b-k	2.2 f-l	2.2 f-l
	Rehani-3	1.4 l	2.4 d-l	2.6 b-k	2.3 e-l	2.0 h-l
	SLB-6	2.1 g-l	3.5 a-d	3.1 a-h	2.8 a-j	2.7 a-k
0.9	Rum	1.6 kl	2.8 a-j	2.5 c-l	2.3 e-l	1.8 j-l
	Rehani-3	1.6 kl	2.4 d-l	2.2 f-l	2.1 g-l	1.8 j-l
	SLB-6	1.9 i-l	3.3 a-f	2.3 e-l	2.4 d-l	2.2 f-l

Values followed by the same letter(s) are not significantly different (p = 0.05) according to LSD

Table 7: Effects of temperature and Water Potential (WP) on root fresh weight (mg/seedling) of three barley cultivars

		Temperature (°C)				
WP (-MPa)	Cultivar	9	15	21	27	33
0	Rum	3.8 t-x¶	19.6 d-m	24.1 d-h	39.0 ab	18.7 d-n
	Rehani-3	4.4 s-x	15.1 g-s	21.7 d-k	36.0-c	27.7 cd
	SLB-6	7.1 o-x	22.5 d-j	22.9 d-h	44.4 a	27.3 c-e
0.3	Rum	3.4 u-x	11.4 j-x	15.7 f-r	22.7 d-i	19.3 d-n
	Rehani-3	3.7 t-x	15.9 f-q	20.7 d-l	26.6 c-f	21.8 d-k
	SLB-6	4.9 p-x	14.8 g-t	25.6 c-g	29.4 b-d	16.3 e-o
0.6	Rum	2.9 wx	13.9 h-w	14.3 h-u	16.0 f-p	6.7 o-x
	Rehani-3	4.0 s-x	11.7 i-x	15.7 f-r	19.7 d-m	18.6 d-n
	SLB-6	4.7 r-x	14.8 g-t	19.7 d-m	22.9 d-h	10.2 l-x
0.9	Rum	2.7 x	6.3 o-x	9.1 m-x	4.7 r-x	4.8 q-x
	Rehani-3	3.5 u-x	7.2 o-x	13.0 h-x	7.4 o-x	5.8 o-x
	SLB-6	4.8 q-x	8.2 n-x	10.3 l-x	10.9 k-x	4.9 p-x

Values followed by the same letter(s) are not significantly different (p = 0.05) according to LSD

cultivars Rum and SLB-6 showed the lowest germination rate at 33°C, while the effect of this temperature on the cultivar Rehani-3 was pronounced only at low water potential levels (-0.6 and-0.9 MPa) (Table 3). The cultivar Rehani-3 had higher germination rate index than the other two cultivars regardless of temperature or water potential.

All cultivars had longest main axis root (MARL) and total root length (TRL) at temperatures 21 and 27°C at all water potentials except the highest level (-0.9 Mpa) (Table 4 and 5). Water stress reduced MARL and TRL of the three cultivars significantly at temperatures 21°C and above (Table 4 and 5). The cultivar SLB-6 had generally higher MARL and TRL than the other two cultivars at all temperatures even though these differences were only significant between SLB-6 and Rum at 21°C for MARL and TRL at-0.3 and for MARL at-0.6 MPa (Table 4). Higher number of seminal roots (SRN) was obtained at temperatures of 15°C and above for all cultivars (Table 6). Water stress had less effect on SRN in the three cultivars at temperatures of 21°C and above. Significant differences for SRN between cultivars were noted only at temperature 27°C for seeds placed in solutions of-0.3 MPa when the cultivar SLB-6 had higher SRN than the other two cultivars.

Root Fresh Weight (RFW) of all cultivars was higher at 27°C in comparison to other temperatures at water potentials 0 to-0.6 MPa (Table 7). Water stress significantly reduced root fresh weight at all temperatures except at 9°C for all cultivars. No significant differences between cultivars were noted for root fresh weight regardless of temperature or water potential.

DISCUSSION

Early high germination across a large range in temperatures may be beneficial for rapid establishments of plants in arid and semiarid regions where soil moisture in the upper soil surface is available for only a short period [9]. The results of this study showed a significant effect of temperature on final germination percentage and rate of germination for the three barley cultivars tested. However, it seems that cultivar Rehani-3 is more tolerant to high temperatures and water stress conditions when compared to the other cultivars tested in respect to germination percentage and rate. Sharma [15] found that germination rate was positively affected by increasing temperature, whereas final germination percentage increased up to a certain limit with increasing temperature.

Temperature and water stress affected the ability of seedlings to develop a root system rapidly after germination, which is critical for successful plant establishment [11]. They found that the optimum temperature range for germination of several Vicia species was lower than that for root growth.

In this study, the optimum temperature was 15°C for final germination percentage and 15 and 21°C for germination rate. The optimum temperatures for early root growth were 21 and 27°C regardless of water stress level. Lowest root growth was noted at 9°C. Lower rates of root growth of wheat (*Triticum aestivum* L.) were obtained at low temperatures of 3 to 10°C [12]. However, water stress had a depressing effect on all studied characters especially at optimum temperatures for growth. These results are in agreement to Soltani *et al*. [16] and Al-Karaki [1] who reported that water stress adversely affected germination. Neverthless, Lafond and Fowler [13] indicated that the effect of temperature on germination was much larger than moisture potential.

Brar *et al*. [9] indicated that since temperature had a strong influence on germination, seeding time should be selected to match expected temperatures required for successful germination at a particular location. They added that early high germination across a large range in temperatures might be beneficial for rapid establishment of the crop in semi-arid warm regions where soil moisture in the upper soil surface is available for only a short period. If seed germination and subsequent root development occurs rapidly, the survival of established seedlings is improved mainly due to the possibility of moisture uptake from greater soil depths [17].

The cultivar Rehani-3 attained the highest final germination percentage and rate than the other two cultivars regardless of temperature or water stress. However, the cultivar SLB-6 had generally higher main axis root and total root lengths than other cultivars regardless of temperature or water potential level. Initial root elongation was found to vary among different genotypes of legume species [9, 11].

In conclusion, seed germination of barley under suboptimal conditions, simulated by different temperatures or water potential in this study, is strongly influenced by cultivar. The cultivar Rehani-3 tended to germinate faster but had slower rooting growth than the cultivar SLB-6 regardless of temperature or water potential level. The cultivar SLB-6 may have better seedling establishment and increased survival ability than the other two cultivars in arid and semiarid regions since its roots could elongate rapidly, thus, ensuring a continuing water supply to the plant. However, additional work is needed to evaluate germination and early seedling growth of these barley cultivars under field conditions.

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Effect of Self, Open and Cross Pollination on Fruit Characteristics of Some Plum Cultivars

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Abstract: This investigation was performed during 2005 and 2006 seasons on three plum cultivars Hollywood, Golden Japanese and Santa Rosa trees grown in loamy soil at Sendyon village, Kalubia governorate, Egypt. The present study was carried out to determine the effects of different types of pollination on fruit physical and chemical characterstics. Data showed that cross pollination of Santa Rosa cv. with Golden Japanese cv. gave the heaviest and largest fruits, followed by Golden Japanese cv. cross pollination with Santa Rosa cv. during the two studied seasons. Also Santa Rosa cv. under cross pollination with Golden Japanese pollen grains yielded fruits with higher dimensions than with self pollination. In case of fruit shape index and firmness, results showed some differences regarding the pollination type and generally cross, open pollination had fruits of round shape, meanwhile fruits resulted from self pollination tended to be oblong shape. Cross pollination of Santa Rosa cv. with Hollywood pollen grains recorded the highest fruit firmness, meanwhile Hollywood cv. with self pollination recorded the lowest firmness during the two studied seasons. Total soluble solids (TSS %), TSS/acid as well as total sugars % were increased at cross pollination of Hollywood cv. with Santa Rosa cv. during the two seasons of study.

Key words: Cultivars% fruit characteristics% Plum % pollination

INTRODUCTION

Plums (*prunus salicina*) are occupying an important share in the total fruit production of Egypt. Plum fruit are mainly marketed as fresh consumption as well as for drying. Also using for canning freezing, Jam and Jelly products [1].

The total area of plum in Egypt reached about 2960 feddans which produced 17148 tons with an average yield of 5.79 tons/fed. according to the census of Egyptian Ministry of agriculture [2].

The majority of plum cultivars are self incompatible and failed to give satisfactory yield in the absence of suitable pollinizers. In addition, cross and open pollination of different plum cultivars resulted in significant increases in fruit set, large and more uniform crops than self pollination. Therefore, to ensure maximum fruit production from plum orchards it is necessary plant cultivars with sufficient overlap in bloom, to permit adequate cross pollination, is necessary.

The effect of pollination on apple fruit weight and size were found by El-Wakee *et al.* [3], Arafat *et al.* [4] and Stino *et al.* [5]. Fruit shap index Miller [6] Khalil [7] and Stino [5], Tss Larry *et al.* [8] El-Sherbini [9] and Stino *et al.* [5], Firmness Arafat *et al.* [4] and Stino *et al.* [5], Acidity, El-Sherbini [9], church and Williams [10] and Stino *et al.* [5] has been studied.

Moreover, the effect of different pollination treatments has been studied in different fruit trees species, such as apricot Burgos *et al. [*11], almond Ahmed *et al.* [12], Ortega *et al.* [13] and on olive Essa [14], Hassan [15], Atawia [16] Milany *et al.* [17] and El-Agamy *et al.* [18].

The main objective of the present investigation was to study the relationship of pollination regime in the studied cultivars on plum fruit characteristics.

MATERIALS AND METHODS

This investigation was performed during 2005 and 2006 seasons on 13 years old trees of three plum cultivars namely, Hollywood, Golden Japanese and Santa Rosa trees were grown in loamy soil at Sendyon village, Kalubia governorate Egypt. These trees were cultivated

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at 5×5 M apart under basin irrigation system. Trees were similar in their vigor and subjected to the same horticultural practices. The trees were randomly selected from each cultivar in each replicate tree 40 shoots were labbled and divided randomly into 4 groups (each of 10 shoots) and examined for either self, open and cross pollination.Three replicate trees were chosen for each cultivar and one tree per replicate to study the pollination treatments.

Pollination studies: In each season of the study and on each cultivar four pollination treatments were made as follows:

- Self pollination (bagging only),
- Open pollination,
- Cross pollination by the other selected cultivars (Hollywood, Golden Japanese and Santa Rosa).

For self pollination (bagging only) flowers were covered with pergamine bags prior to flower opening. This was done before flower opening for about 3-4 days to 15 days after full bloom. In open pollination the flowers were left under the natural conditions of the orchard. Meanwhile, cross pollination of each cultivar was done when flowers were at balloon stage were hand emasculated; covered with pergamine bags till reached the stage of anthesis and then pollen grains of the two male parents applied to the stigma of emasculated flowers.

Fruit physical characteristics: Sample of 20 mature fruits were taken from each replicate tree of each pollination type and the following characteristics were determined:

- Average fruit weight (g),
- Average fruit volume (cm^3),
- Specific gravity (gm cm^{-3}),
- Average fruit length and diameter (cm),
- Fruit shape index (L/D),
- Fruit firmness was determined as (Lb/$inch^2$) by using taylor pressure tester 5/16 inch plunger.

Fruit chemical characteristics:

- Total soluble solids Tss % of fruit juice was measured by using a, Hand Refractometer,
- Fruit acidity, the percentage of total acidity in fruit juice was determined as malic acid according to A.O.A.C. [19],
- Total soluble solids/acid ratio,
- Total soluble sugars, was determined according to smith [20] in methanolic extract using the phenol sulphoric acid methods and the percentage was calculated on dry weight basis.

Statistical analysis: A completely randomized block design was used and the obtained data were subjected to analysis of variance and Duncan's multiple range test used to differentiate means Duncans [21].

RESULTS AND DISCUSSION

Fruit physical and chemical characteristics

1 - Physical characteristics

Fruit weight, volume and specific gravity: Results in Table 1 show that the values of fruit weight and volume of three plum cultivars Hollywood, Golden Japanese and Santa Rosa under different pollination treatments (self, open and cross) during 2005 and 2006 seasons. It is evident that, Santa Rosa under cross pollination with Golden Japanese gave the heaviest and largest fruits followed by Golden Japanese cross pollination with Santa Rosa, during the two successive seasons.

Concerning specific gravity data presented in Table 1 showed that specific gravity was not significantly affected by different pollination treatment in the first season. Meanwhile in the second one Santa Rosa open pollination recorded the highest specific gravity while Hollywood cross with Santa Rosa pollen grain recorded the lowest specific gravity.

These results are agreement with those obtained by El-Wakeel *et al.* [3] on apple, Stino *et al.* [5] on apple and Kilany *et al.* [17] on olive.

Fruit length and width: The present data in Table 2 indicated that, cross pollination of Santa Rosa cv. with Golden Japanese pollen grains yielded fruits with higher dimensions as compared with self pollination.

The above results goes in line with those obtained by Essa [14], Hassan [15], Atawia [16], El-Kilany *et al.* [17] on olive they demonstrated that fruit diamension were significantly affected by different pollination treatments.

Fruit shape index and firmness: Data presented in Table 3 showed that fruit shape index were affected by different pollination types and generally cross and open pollination had round fruit shape, meanwhile those fruits resulted in from self pollination tended to be oblong shape.

Table 1: Effect of self, open and cross-pollination on fruit weight, volume and specific gravity of some plum cultivars in 2005 and 2006 seasons

		Fruit weight (g)		Fruit volume (cm^3)		Specific gravity	
Cultivars	Treatments	2005	2006	2005	2006	2005	2006
Hollywood	Self Pollination	32.00I	34.03f	31.67h	33.50g	1.07a	1.01b
	Open Pollination	37.17gh	39.33e	36.10g	38.70ef	1.00a	1.01b
	Cross with Golden Japanese	44.57de	46.40cd	42.57ef	44.17cd	1.47a	1.05ab
	Cross with Santa Rosa	44.90de	45.33d	44.23de	45.67bc	1.01a	0.99b
Golden Japanese	Self Pollination	40.43fg	39.20e	39.67f	38.27ef	1.01a	1.02ab
	Open Pollination	45.77cde	47.67bcd	45.00cde	46.00bc	1.01a	1.03ab
	Cross with Hollywood	47.83bcd	49.63bc	46.97bcd	48.00abc	1.01a	1.03ab
	Cross with Santa Rosa	50.20ab	51.50ab	49.20ab	49.50ab	1.01a	1.03ab
Santa Rosa	Self Pollination	36.37h	37.87e	35.90g	36.33fg	1.00a	1.03ab
	Open Pollination	43.20ef	44.83d	40.33f	41.33de	1.02a	1.07a
	Cross with Hollywood	48.80bc	51.27ab	48.10abc	49.27ab	1.01a	1.04ab
	Cross with Golden Japanese	53.10a	54.13a	51.17a	51.67a	1.03a	1.04ab

Table 2: Effect of self, open and cross-pollination on fruit length and width of some plum cultivars in the 2005 and 2006 seasons

		Fruit length (cm)		Fruit width (cm)	
Cultivars	Treatments	2005	2006	2005	2006
Hollywood	Self Pollination	3.76bcd	3.86c	3.56e	3.70d
	Open Pollination	3.93abcd	4.06bc	3.96cd	4.03bc
	Cross with Golden Japanese	4.03abcd	4.10bc	4.16abcd	4.23ab
	Cross with Santa Rosa	4.06abcd	4.26ab	4.13abcd	4.23ab
Golden Japanese	Self Pollination	3.70cd	3.90c	4.13abcd	4.26ab
	Open Pollination	3.96abcd	4.16abc	4.26abc	4.36a
	Cross with Hollywood	4.13abc	4.30ab	4.26abc	4.40a
	Cross with Santa Rosa	4.06abcd	4.16abc	4.33ab	4.43a
Santa Rosa	Self Pollination	3.63d	4.03bc	3.83de	3.93cd
	Open Pollination	4.13abc	4.16abc	4.03bcd	4.23ab
	Cross with Hollywood	4.20ab	4.30ab	4.30abc	4.26ab
	Cross with Golden Japanese	4.33a	4.43a	4.43a	4.50a

Table 3: Effect of self, open and cross-pollination on fruit shape index and firmness of some plum cultivars in the 2005 and 2006 seasons

		Fruit shape index		Fruit firmness Ib/inh2	
Cultivars	Treatments	2005	2006	2005	2006
Hollywood	Self Pollination	1.05a	1.04a	10.17f	10.20h
	Open Pollination	0.98ab	1.00ab	10.20f	10.33gh
	Cross with Golden Japanese	0.96bc	0.96abc	10.47ef	10.50efg
	Cross with Santa Rosa	0.95bc	1.00ab	11.50ab	11.57a
Golden Japanese	Self Pollination	0.89c	0.91c	10.20f	10.40fg
	Open Pollination	0.92bc	0.94bc	10.57de	10.57def
	Cross with Hollywood	0.95bc	0.97abc	10.70de	10.80c
	Cross with Santa Rosa	0.93bc	0.93bc	11.17bc	11.30b
Santa Rosa	Self Pollination	0.94bc	1.02ab	10.90cd	10.70cd
	Open Pollination	0.98ab	0.98abc	10.63de	10.60de
	Cross with Hollywood	0.97abc	1.00ab	11.57a	11.60a
	Cross with Golden Japanese	0.97abc	0.98abc	11.47ab	11.47b

Table 4: Effect of self, open and cross-pollination on TSS% and Acidity of some plum cultivars in the 2005 and 2006 seasons

		TSS %		Acidity %	
Cultivars	Treatments	2005	2006	2005	2006
Hollywood	Self Pollination	13.53c	13.80b	1.00bc	1.03ab
	Open Pollination	13.93abc	14.33a	0.86cd	0.96b
	Cross with Golden Japanese	14.23ab	14.53a	0.90cd	0.86c
	Cross with Santa Rosa	13.87abc	14.63a	0.76d	0.83cd
Golden Japanese	Self Pollination	11.83e	12.43d	1.13ab	1.03ab
	Open Pollination	12.00de	12.93c	1.20a	1.00ab
	Cross with Hollywood	12.50d	12.67cd	1.13ab	1.06a
	Cross with Santa Rosa	12.50d	14.47a	1.10ab	1.03ab
Santa Rosa	Self Pollination	14.47a	13.80b	0.83d	0.76d
	Open Pollination	13.67bc	13.93b	0.76d	0.83cd
	Cross with Hollywood	13.73bc	14.33a	0.80d	0.83cd
	Cross with Golden Japanese	14.07abc	14.67a	0.90cd	0.86c

Table 5: Effect of self, open and cross-pollination TSS/acid ratio and Total sugars of some plum cultivars in the 2005 and 2006 seasons

		TSS/acid ratio		Total sugars %	
Cultivars	Treatments	2005	2006	2005	2006
Hollywood	Self Pollination	13.60e	13.37c	8.63de	8.83def
	Open Pollination	17.16bcd	14.87b	9.20bc	9.20d
	Cross with Golden Japanese	15.27de	16.83a	9.96a	10.13ab
	Cross with Santa Rosa	18.17a	17.63a	10.30a	10.40a
Golden Japanese	Self Pollination	10.50f	12.03d	8.60de	8.66ef
	Open Pollination	10.03f	12.93cd	8.33e	8.46f
	Cross with Hollywood	11.03f	11.90d	8.76d	8.93de
	Cross with Santa Rosa	11.40f	14.03bc	9.96a	10.17ab
Santa Rosa	Self Pollination	17.40abc	18.07a	8.93cd	9.60c
	Open Pollination	17.90ab	16.77a	9.43b	9.96bc
	Cross with Hollywood	17.20abc	17.23a	10.20a	10.27ab
	Cross with Golden Japanese	15.77cd	16.97a	8.78d	8.60ef

Regarding fruit firmness data presented in the same table cleaed that fruit firmness was significantly affected with different pollination treatments.

Santa Rosa cv. Cross with Hollywood pollen grains recorded the highest fruit firmness meanwhile Hollywood under self pollination recorded the lowest fruit firmness during two successive seasons. The above results agree with those obtained with Miller [6] and Khalil [7], Arafat *et al.* [4] and Stino *et al.* [5].

2 - Chemical characteristics

Total Soluble Solids (TSS %) and acidity: Data in Table 4 revealed that, Santa Rosa plum fruit under self pollination recorded the highest Juice TSS % in the first season, meanwhile in the second one Santa Rosa plum fruit cross with golden Japanese recorded the highest TSS %. On the other hand self pollination Golden Japanese plum fruit recorded the lowest fruit TSS % during both seasons, respectively.

As for total acidity % of fruit Juice, data presented in Table 4 revealed that, Santa Rosa fruit Juice under self and open pollination recorded the significantly lowest fruit Juice acidity % during the first and second seasons, respectively.

TSS/acid ratio and total sugars %: Data in Table 5 indicted that Hollywood fruit which cross pollinated with Santa Rosa gave the highest percentage of TSS/acid ratio during the first season. Meanwhile, Santa Rosa self pollinated recorded the highest range in this respect during the second season.

Concerning total sugars data in Table 5 showed that, Santa Rosa pollens induced higher total sugars values when used as a pollinizer for Hollywood cultivar during both season, respectively. On the other hand, open pollination in golden Japanese induced the lower

percentage of total sugars in first and second seasons, respectively.

Similar results were reported by El-Sherbini [9], church and Williams [10], Stino *et al.* [5] and El-Agamy *et al*. [18]. TSS, acidity and total sugar were affected with different pollination treatments.

Generally, it can be concluded from the obtained results, study, that cross and open pollination were more effective and resulted in an improved fruit physical and chemical characteristics while self pollination ranked later in this respect.

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Total Electrophoretic Band Patterns of Some Onobrychis Species Growing in Turkey

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Abstract: The aim of this study was to investigate interspecific variations in Sections Lophobrychis, Onobrychis, Hymenobrychis of genus Onobrychis by sodium dodecyl sulphate polyacrilamyde gel electrophoresis (SDS-PAGE) technique. In this study total eight species collected from different regions of Turkey have been studied for the analysis of seed storage protein profiles to examine their relationship. Electrophoretic data were documented by using a gel documentation system and analysed by using Quantity 1-D analysis software and also the dendogram were formed in UPGAMA. Studied species of genus Onobrychis sections Lophobrychis, Onobrychis, Hymenobrychis cluster together on the basis of seed protein similarities as designed by previous morphological classification. The formed dendogram from SDS-PAGE analysis showed that all studied species constituted two clusters. The first one consisted of *O. caput-galli, O. aequidendata, O. fallax, O. armena, O. viciifolia* and second one by *O. hypargyrea, O. galegifolia* and *O. cappadocia*. Protein amounts of all species were also found to be between 49.864-56.966 µg ml^{-1}.

Key words: *Onobrychis* % SDS-PAGE % total protein % legume seeds % similarity matrix % UPGAMA

INTRODUCTION

The genus *Onobrychis* Miller extends from the mediterranean region to Central Asia. The majority of the species are restiricted to North-west Asia, especially Iran and Anatolia, making this area the main centre of genetic diversity of the genus [1]. Many species are exploited as high protein fodder plants for several animals and they play an important role in enrichment of soil, increasing the nutritive value of drought-resistant pasture [2].

Onobrychis an extremely difficult genus with many of the worst problems in Anatolia-one of the main centres of the genus [3]. The taxonomy of the genus continues to be a subject of much confusion, mainly because of the different approaches to species delimitation, resulting in varying numbers of recognized species [4]. Recently Yildiz *et al*. [1] suggested based on fruit morphology genus *Onobrychis* consist of 170 species, eight sections and two subgenera. In Flora of Turkey (volume 3) many of the species dealth with cannot be defined or keyed out satisfactory and numerous taxonomic problems throughtout the genus await solution and genus is represented with 54 species and divided into five sections [3-5]. Grain legumes are widely recognized as important sources of food and feed proteins. In many regions of the world, legume seeds are the unique supply of protein in the diet [6]. Legume seeds have a high level of protein content, ranging from approximately 200 to 400 g kg^{-1} as compared to other sources of plant proteins [7]. Legume seeds are highly stable, unaffected by environmental conditions. Therefore electrophoretic techniques for total seed protein analysis have been recognized as a valid source of taxonomic evidences and were used to adress taxonomic relationships at the generic and specific levels [8]. In literatures, most limited studies have been performed on the genus *Onobrychis* and as far as the literature search show electrophoretic analysis and protein amounts of species (exclude from *O. viciifolia)* from genus *Onobrychis* examined in the present study has not yet been investigated.

The objective of the present study was to investigate interspecific variations in Sections Lophobrychis, Onobrychis, Hymenobrychis by sodium dodecyl sulphate polyacrilamyde gel electrophoresis (SDS-PAGE)

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Table 1: Localities of investigated *Onobrychis* species

Species	Section	Province	Locality
O. caput-galli (L.) Lam.	Lophobrychis	Manisa	Near to Kula dam lake
O. aequidentata (Sibth. and Sm.) d'Urv	"	Manisa	Salihli
O. fallax Freyn and Sint.	Onobrychis	Elazig	University campus
O. armena Boiss. and Huet	"	Usak	Usak-Akarca road
O. viciifolia Scop.	"	Usak	Banaz
O. hypargyrea Boiss.	Hymenobrychis	Gediz	Gediz-Simav road
O. galegifolia Boiss.	"	Elazig	Harput
O. cappadocia Boiss.	"	Elazig	University campus

technique. In this study total eight species collected from different regions of Turkey have been studied for the analysis of seed storage protein profiles to examine their relationship. Protein amounts of samples were also determined.

MATERIALS AND METHODS

Dry seeds of *Onobrychis* species were collected from various areas of Turkey. Details about the seed materials are given in Table 1.

Seed proteins were extracted as described by Jha and Ohri [9]. Seed coats were removed prior to extraction and cotyledons were obtained. These were homogenised in 0.1M Tris-HCl buffer (pH: 7.5). Total protein was extracted after centrifugation at 17.600 g for 20 min at 4°C and supernatants were used for analysis. Proteins in the supernatants were quantified using Bio-Rad DC protein assay (Bio-Rad Laboratories, UK) and on the gel, Fermentas (116.0 kDa (kilodalton), 66.2 kDa, 45 kDa, 35 kDa, 25 kDa, 18.4 kDa) used as marker. The samples were boiled for 5 minutes prior to loading, then equal amount of each sample was loaded on to the 12% SDS-PAGE [10]. Electrophoresis was performed in the Protean II electrophoresis cell (Bio-Rad Laboratories, UK) at 20 mA until the bromophenol dye (BDH Laboratory Supplies Poole, England) front had reached the bottom of the gel. The gels were stained in Coomassie Brilliant Blue (Sigma Aldrich Chemie, Germany) solution for 30 min at 67 °C and destained in destaining solution for 3-4 h at 67°C to visualise the proteins.

Statistical analysis: Electrophoretic data were documented by using a gel documentation system (Bio-Rad, USA) and analysed by using Quantity 1-D analysis software and also the dendogram were formed with 4.0% tolerance in UPGAMA (Unweighed Pair-Group Arithmetic Mean). Also, similarity matrix was constructed by Dice coefficient using Quantity 1-D (Bio-Rad) and expressed as percentages.

RESULTS AND DISCUSSION

Many studies based on the electrophoretic analysis of seed proteins have been used to examine genetic variability and systematic problems in several legumes such as genus *Astragalus* [11], genus *Lupin* [12], genus *Pisum* [9], genus *Phaseolus* [13], genus *Lathyrus* [14-16]. The differences among species were observed and all eight species were clearly identifiable from the protein patterns. The total seed protein banding patterns of eight species were illustrated in Fig. 1. Additionally, protein amounts of studied species were given in Table 2 and also similarity matrix were given Table 3. Protein amounts of all species were also found to be between 49.864-56.966 µg ml^{-1}.

The all studied species of genus Onobrychis sections Lophobrychis, Onobrychis, Hymenobrychis cluster together on the basis of seed protein similarities as designed by previous morphological classification. The formed dendogram from SDS-PAGE analysis showed that all studied species constituted two clusters (Fig. 2). The first one consisted of *O. caput-galli*, *O. aequidendata*, *O. fallax*, *O. armena*, *O. viciifolia*

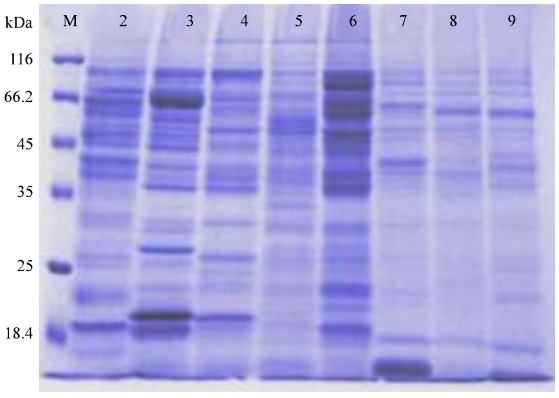

Fig. 1: SDS-PAGE of total seed proteins in eight taxa. M: Marker; 1: *O. caput-galli*; 2: *O. aequidentata*; 3: *O. fallax*; 4:*O. armena*; 5: *O. viciifolia*; 6: *O. hypargyrea*; 7: *O. galegifolia* 8: *O. cappadocia*

Table 2: Protein amounts of investigated *Onobrychis* species

Species	Protein amounts (µg mlG¹)
O. caput-galli (L.) Lam.	56.966
O. aequidentata (Sibth. and Sm.) d'Urv	54.294
O. fallax Freyn and Sint.	51.251
O. armena Boiss. and Huet	54.531
O. viciifolia Scop.	54.971
O. hypargyrea Boiss.	53.178
O. galegifolia Boiss.	49.864
O. cappadocia Boiss.	51.724

Table 3: Similarity matrix of investigated *Onobrychis* species

	2	3	4	5	6	7	8	9
2	100.0	48.2	37.2	37.8	45.8	34.7	35.7	34.2
3	48.2	100.0	29.7	47.5	47.3	33.9	22.8	25.0
4	37.2	29.7	100.0	44.2	49.4	47.0	42.3	46.4
5	37.8	47.5	44.2	100.0	58.3	39.9	32.5	27.1
6	45.8	47.3	49.4	58.3	100.0	43.6	36.8	33.8
7	34.7	33.9	47.0	39.9	43.6	100.0	54.8	55.0
8	35.7	22.8	42.3	32.5	36.8	54.8	100.0	62.9
9	34.2	25.0	46.4	27.1	33.8	55.0	62.9	100.0

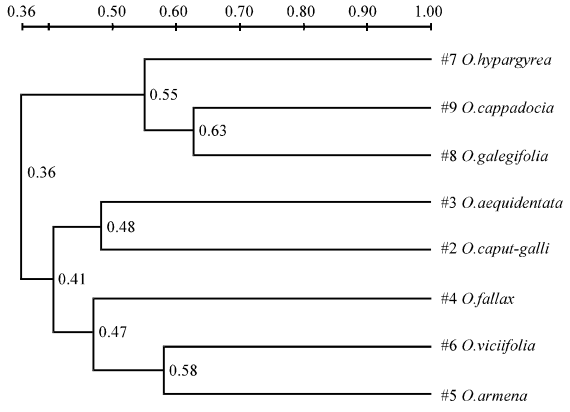

Fig. 2: Dendogram of *Onobrychis* species based on total seed protein profiles

second one by *O. hypargyrea*, *O. galegifolia and O. cappadocia. O. armena* and *O. viciifolia* found to have higher similarity to each other than *O. fallax* when the results of cluster I were compared. Also *O. caputgalli* and *O. aequidendata* which are the members of section Lophobrycis closer to each other rather than the other members of cluster I. Similarly, in cluster II, *O. galegifolia* and *O.cappadocia* closer to each other than *O. hypargyrea* which is partly differ from these two species of cluster II.

In a study chromosomal criteria and phylogenetic implications in the genus *Onobrychis* done by Abou-El-Enain [2] revealed that two species (*O. caput-galli* and *O. aequidendata*) from section Lophobrychis, it can be concluded that the recorded variation in chromosome numbers in each of *O. caput-galli* and *O. aequidendata*, can be referred to the differences in their taxonomic delimitation, at least at the subspecies level. Abou-El-Enain [2] also suggested that section Lophobrychis has a comparatively highly derived organization and can be considered as a heterogenous unit in the genus *Onobrychis.* But results from our present study showed that *O. caput-galli* and *O. aequidendata* have similar total band profiles especially between 116 and 35 kDa. Therefore, further investigation of phylogenetic relationships between these species needed by analysis of genetic variations between the species, such as analysis of subprotein fractions (albumins, globulins etc.) and RAPD studies.

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Vertical Distribution of Soil Organic Carbon in Agroecosystems of Songliao Plain along a Latitudinal Gradient

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Abstract: Soil Organic Carbon (SOC) is related closely to the atmospheric CO_2 and soil quality. Understanding the regional and vertical distribution of soil organic carbon is a key to predict and simulate the influences of climate, global change and human activities on the terrestrial carbon cycling. Soil organic carbon and SOC pool in profile extending to a depth of 100 cm were assessed in agroecosystems of Songliao Plain (Northeast China) along a latitudinal gradient. The results indicated that the concentration of SOC decreased with the increasing of soil depth and significant depth effects were observed in Hailun, Harbin, Gongzhuling and Changtu sampling sites ($p<0.05$), especially between topsoil and lower layers. SOC storage in profiles of every sampling site presented also decreasing with the increasing of depth. Significant correlations either between SOC concentration and latitude or between SOC pool and latitude were observed in all layers ($p<0.05$), but their correlation coefficients decreased with the increasing of depth. Human activities and the latitudinal transect resulting in different climate and soil types may be the main factors influencing the vertical and latitudinal distribution of soil organic carbon.

Key words: Soil organic carbon % vertical distribution % latitudinal gradient % agroecosystem % songliao plain

INTRODUCTION

Soil Organic Carbon (SOC) is related closely to the atmospheric CO_2 and soil quality [1-3]. The interaction between global climate change and dynamics of soil carbon is co-existence in an ecosystem. At present time, studies on soil carbon have been an important question, especially after the Kyoto-Protocol was adopted. Moreover, soil scientists all over the world have focused on the research of soil carbon because of its dynamic change leading to global environmental problems [4, 5]. Many studies have pay attention to the questions of soil carbon pool and its influencing factors [6-10] and soil scientists have made attempts to estimate the amount of C stored in global soils. Among these results, it is depending that Post *et al*. [11] and Eswaran *et al*. [12] reported the quantity of soil carbon pool was between 1395 and 1576 Gt in global soil. The SOC content of soil in China has been also studied in several regions, such as East China, Southeast China and Northeast China [7, 13-16].

Agroecosystem as the important part of terrestrial ecosystems is the valuable proportion in study of C cycling. In agroecosystems, plowing leads to dramatic losses of SOC through intensive soil disturbance that disrupts soil moisture and enhance decomposition, in addition to accelerating soil erosion [17]. Cultivation also redistributes organic C deeper in the profile through the mixing action of tillage implements [18]. Lal *et al*. [19] reported that SOC pool in American farmland soil decreased greatly after reclamations without restraint. However, many studies have proved that scientific agricultural management practices can increase SOC pool in farmland soils [20]. Therefore, SOC sequestration in agoecosystems is very important to mitigate global greenhouse effect.

There have been studies on the vertical distribution of soil organic carbon [7, 15, 21, 22]. These studies showed that SOC concentration was highest in topsoil. Jiang *et al*. [15] also reported that SOC content in the profiles decreased from upper layer to deeper layer. Lemaire *et al*. [23] reported that the majority of studies on

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soil carbon were limited in cultivated layers and studies on the distribution of soil carbon in different soil types are meaningful for local soil resource conservation as well as atmospheric environment protection [13]. However, little is known about the vertical distribution of soil organic carbon in Northeast China. Therefore, understanding the regional and vertical distribution of soil carbon is a key to predict and simulate the influences of climate, global change and human activities on the terrestrial carbon cycling. The objective of this research was to investigate the profile distribution of soil carbon in the typical croplands of Songliao Plain along a latitudinal gradient.

MATERIALS AND METHODS

Study site: A total of seven sites were chosen along a latitudinal transect from north (Hailun) to south (Dashiqiao) in corn fields of Songliao Plain, Northeast China (Table 1). No frost period and mean annual precipitation (MAT) in every sampling site increase with the latitude increasing and mean annual temperature (MAP) varied little among sampling locations which had the same soil types. There are two soil types including black soil and brown soil. The crop types of all locations were corn (*Zea mays* L.) and the tillage system belongs to conventional tillage.

Soil sampling and analysis: In October 2005, at the end of the harvesting, soil samples were collected by using soil auger at each site along the latitude gradient. At each sampling site, four replicates were taken to a depth of 1 meter at 0-20, 20-40, 40-60, 60-80 and 80-100 cm depth intervals and every sample was composed of four mixing samples. Each soil sample was placed in individual plastic bag and then brought to laboratory. All the soil samples were sieved through a 2 mm sieve in order to eliminate the plant roots. The following analyses were undertaken:

SOC: Sub-samples from each sample were grounded to power passing through 0.149 mm sieve. Then, the SOC contents were determined by the dry combustion method using a Vario ELIII Elemental Analyzer.

Soil bulk density: Soil samples were oven-dried at 105°C for 24 h and weight a standard volume of field soil [24].

Soil texture: Using the pipette method, dispersion of soil sample using chemical dispersant, soil suspension is poured on to a fine sieve with 0.05 mm for separating out the sand fraction; the silt and clay fractions are washed through the sieve into a sedimentation cylinder. This suspension is thoroughly stirred and allowed to settle. The clay content is determined by drying a constant volume suspension extracted with a pipette at a certain depth after 7 h [24].

Storage of SOC: It was calculated by the following formula [15]:

$$SOC_s = \sum_{i=1}^{n} (1 - \theta_i\%) \times \rho_i \times C_i \times T_i / 10 \quad (1)$$

SOC_s: the storage of soil organic carbon (t C ha^{-1})
θ_i: the volumetric percentage (%) of fragments 2 mm
ρ_i: the bulk density (g cm^{-3}) of layer i
C_i: the content of carbon (g kg^{-1}) in layer i
T_i: the thickness (cm) of layer i

All the data obtained in the study were subjected to statistical analysis of variance (ANOVA) and Pearson correlation analysis. Differences at the $p<0.05$ level were considered to be significant.

RESULTS

Concentrations of SOC: Concentrations and vertical distribution of SOC in cropland soil along latitudinal

Table 1: Description of sampling sites

Sampling site	Latitude	Longitude	Soil type	No frost Day/a	MAP mm	MAT°C
Hailun	47°27'N	126°55'E	Black soil	116	550	1.5
Harbin	45°43'N	126°45'E	Black soil	120	523	3.6
Dehui	44°32'N	125°45'E	Black soil	138	520	4.4
Gongzhuling	43°31'N	124°48'E	Black soil	135	563	5.6
Changtu	42°41'N	124°02'E	Brown soil	148	654	6.7
Shenyang	41°31'N	123°22'E	Brown soil	183	650	7.5
Dashiqiao	40°41'N	122°34'E	Brown soil	173	657	8.9

Notes: MAP-mean annual precipitation; MAT-mean annual temperature

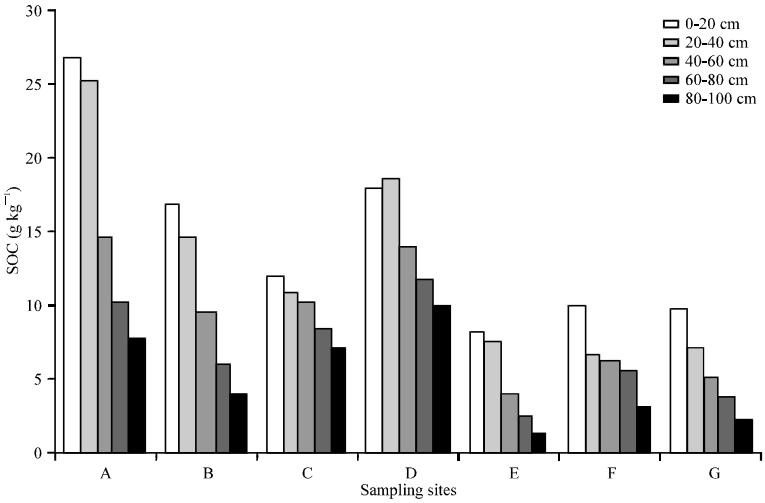

Fig. 1: Concentrations of SOC in soil profiles at different locations
A: Hailun; B: Harbin; C: Dehui; D: Gongzhuling; E: Changtu; F: Shenyang; G: Dashiqiao

Table 2: Profile distribution of SOC storage (t C ha^{-1}) at various depth along different latitude

Depth (cm)	Hailun	Harbin	Dehui	Gongzhuling	Changtu	Shenyang	Dashiqiao	p values[b]
0-20	54.3	35.2	24.6	38.6	18.1	22.7	21.2	<0.01
20-40	54.8	33.1	23.4	41.0	17.5	15.6	15.1	<0.01
40-60	34.1	21.8	22.8	32.2	9.4	14.3	11.1	<0.01
60-80	24.0	13.6	18.2	27.5	5.9	12.9	8.5	<0.01
80-100	18.8	9.0	15.7	23.8	3.7	7.2	5.1	<0.01
p values[a]	<0.01	<0.01	<0.05	<0.05	<0.01	<0.01	<0.01	
0-100	186.0	112.7	104.7	163.0	54.6	72.6	61.0	<0.01

Notes: p values[a] refer to vertical differences between the soil layers, p values[b] refer to differences between the different sampling sites

transect at different locations are shown in Fig. 1. SOC concentrations varied obviously in different locations and there were obvious stratifications of SOC in soil profiles.

The vertical distribution of SOC in every sampling site exhibited the same trends that the concentration of SOC decreased with the depth increasing. The concentration of SOC was higher in every layer of black soils (Hailun, Harbin, Dehui and Gongzhuling) than in the same layer of brown soil (Changtu, Shenyang and Dashiqiao). The result of statistical analyses indicated that there were significant differences of SOC between layer 0-40 cm and layer 40-100 cm in Hailun, Harbin, Gongzhuling and Changtu sampling sites ($p<0.05$).

Storage of SOC: Generally, the volumetric percentage of fragments 2 mm in cultivated soil could be overlooked. The storage of SOC decreased with depth increasing was observed (Table 2). In other words, there was accumulation phenomenon in the upper layers. Significant differences of SOC were observed among different soil layers and among different sampling locations along the different latitude ($p<0.05$).

SOC reservoir calculated to 1 m soil depth at every sampling location was presented in Table 2. SOC reservoirs ranged widely from 54.6 to 186 t C ha^{-1} at different sampling sites (Table 2). Statistical analysis of variance indicated there was significant difference of SOC storage among sampling locations ($p<0.01$).

Correlations between SOC and latitude: The Pearson correlation analysis indicated that there was significant correlation either between SOC and latitudes or between storage of SOC and latitudes (Table 3). Both SOC concentration and SOC pool (SOC_s) increased with the increasing of latitude, but the correlated coefficients decreased with the increasing of soil depth. However, SOC concentration in 0-40 cm layers at Gongzhuling site was higher than at Harbin and Dehui sites.

Table 3: Correlations between the vertical content of SOC and the latitude

	SOC		SOC_s	
Depth (cm)	Liner equation	Correlated coefficients	Liner equation	Correlated coefficients
0-20	Y = 2.3 X-87.3	R = 0.84**	Y = 4.4 x-163.8	R = 0.84**
20-40	Y = 2.5 X-94.6	R = 0.82**	Y = 5.4 x-209.1	R = 0.87**
40-60	Y = 1.4 X-50.6	R = 0.69**	Y = 2.9 x-109.4	R = 0.79**
60-80	Y = 0.9 X-32.9	R = 0.56**	Y = 1.8 x-65.1	R = 0.67**
80-100	Y = 0.8 X-28.5	R = 0.49*	Y = 1.6 x-61.2	R = 0.68*

Notes: Y is SOC concentration and SOC pool (SOC_s); X is latitude; *, **denote 0.05 and 0.01 correlation levels respectively; R is correlated coefficient

Relationship of SOC with soil texture and climate: The content of SOC was positively correlated with soil clay particles content ($r = 0.51$, $p<0.01$) and negatively correlated with the mean annual temperature ($r = -0.56$, $p<0.01$) and no frost period ($r = -0.52$, $p<0.01$).

DISCUSSION

The SOC concentration depended on the balance between organic matter input and its loss from soil. Litter fallen to the soil surface and turnover of fine roots were regarded as the main pathways of SOC input [7]. In addition, the application of organic and chemical fertilizers was also a main pathway of SOC input to soil in agricultural ecosystem. In topsoil, the application of fertilizers resulted in the increasing of quantity of litter returning to soil which made the topsoil with higher SOC concentration. In addition, in cultivated layers, microbes and roots produced a lot of secretions and metabolized materials. All these factors might be result in the SOC concentrations higher in the soil surface than in the deeper layers. Jiang's research about the profile distribution of SOC in different land-use types showed that SOC concentration in the profile decreased with the increasing of depth and they considered the plant cycling above soil surface, eluviation and human activities as the main factors influencing the distribution of SOC in profile [15]. Omonode and Vyn [21] also reported similar results that SOC was highest in the soil surface layer and decreased slowly with the increasing of depth at Maryland and Iowa croplands. By analysis soil particle size composition, we found that clay particle content was relatively higher in topsoil and was positively correlated with SOC in the present study. Arriaga and Lowery [25] also reported there was an important interaction between organic materials and clay particles in the top 20 cm of the soil profile and this interaction results in greater organic C contents in the soil profile where there was greater clay content. In addition, most organic matter is decomposed within the top 10 cm of soil yielding a soluble portion, which migrates to a certain extent towards deeper soil horizons and resulted in SOC accumulating in deeper layers. Hence, the SOC accumulated in the topsoil in the north and can not be complemented immediately in lower soil layers, which resulted in SOC decreasing rapidly in layer 40-60 cm. However, higher precipitation resulted in strong eluviations in the soil of south, SOC decreased slowly with the increasing of depth.

Climatic conditions influence soil biological and physical processes driving soil organic matter dynamic change [26]. In the process of SOC formation and decomposition, temperature and precipitation are the main influencing factors [10]. Soil temperature and no frost days could influence microbial activity and growth periods of corn, which affect mineralization of SOC and the quantity of secretions. SOC density would decrease with temperature increasing [27]. Because of the temperature and no frost period decreasing from south to north, the decomposition intensity of microbe might decrease and the growth periods of corn increased which could increase the secretion of roots. Finally, SOC accumulated in north soil. Via the Century model, Gao *et al*. [28] reported that SOC in Northeast China decreased from north to south in the black soil region. In different climate region, management practices were sensitive to climate in the following order from largest to smallest changes in SOC: tropical moist>tropical dry>temperate moist>temperate dry [17]. Therefore, under the same condition of management practices, SOC lost easily in south soil. To find the effect of soil type on SOC concentrations, Liu *et al*. [22] showed that soil samples under Chernozem have the highest SOC concentration and those under Aeolian have the lowest SOC concentration. In the present study, the SOC content was higher in black soil than that in the brown soil.

In addition, SOC is sensitive to human disturbance under the changing climate. The soil had been cultivated for longer period in the south than in the north and

therefore there had been more SOC lost in south soil, which may be one of the factors affecting latitudinal distribution of SOC [29]. According to the simulating of SOC variation in black soil under different soil managements, the SOC in black soils in Northeast China has been close to a stable equilibrium state, reflecting that there had been a balance between roots input and mineralization output in corn field and SOC content in the north of Northeast China may continue to decrease for a certain time due to the relative shorter history of reclamation [16]. This may also explain SOC content increasing with the latitude.

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Foliar Sprays of Potassium Dihydrogen Phosphate and Their Impact on Yield, Fruit Quality and Controlling Powdery Mildew Disease of Thompson Seedless Grapevines

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Abstract: This investigation was carried out during two successive seasons (2005 and 2006) and included two experiments, the first one (field experiment) was carried out to improve yield and fruit quality of Thompson seedless grape grown under sandy soil condition through foliar sprays of potassium dihydrogen phosphate (KH_2PO_4) at 1 and 1.5% concentrations which sprayed at different periods (every 10, 20 or 30 days). The second experiment (pathological trails) was carried out to study the effect of different potassium dihydrogen phosphate concentrations on spore germination, germ tube length of grape powdery mildew fungi and their diseases infection. Results indicated that, using potassium dihydrogen phosphate as a foliar sprays had a positive effect on leaf mineral content, yield weight and fruit quality of Thompson seedless grapevines specially when sprayed at 1% concentration every 10 days or 1.5% every 20 days from the beginning of April till end of July, such treatments considered the promising under field conditions. On the other hand, a complete inhibition (100%) was recorded with 2.5% concentration of KH_2PO_4 as a tested salt against fungi. Spraying detached leaves with KH_2PO_4 (2.5%) 24 h. before inoculation was more effective than those of 24 h. after inoculation in decreasing values of infection parameters (disease incidence and severity) for powdery mildew, therefore, it was as effective as the fungicide Afugan.

Key words: Thompson seedless grapevines % potassium dihydrogen phosphate % yield % fruit quality % mineral contents % powdery mildew disease

INTRODUCTION

It is well known that most of the new reclaimed area in Egypt are planted with fruit trees especially grapes which considered the second fruit crop in Egypt. The area of the vineyards has increased rapidly through the last few years and reached about 160,005 feddans (one feddan = 4200m^2) (according to the statistics book of Ministry of Agriculture and Land Reclamation, 2005). In Egypt, Thompson seedless grape (*Vitis vinifera*, L.) is considered the leader of grape cultivars and consumed mainly fresh as table grape, so improving fruit quality is a major factor affecting marketing request. Grapes grown under sandy soil conditions have a problem of low productivity due to poor fertility of such soils. Thus, it is highly needed to treat in ways that lead to increase production and improve quality. Many previous studies revealed that P and K sprays especially at form of potassium dihydrogen phosphate enhanced nutritional status and improved yield and quality of different fruit crops. These studies were supported by Shawky *et al.* [1], Abd El-Migeed *et al.* [2] on oranges, Ibrahim *et al.* [3], El-Fangary [4] on mandarin and Saleh and Abd El-Monem [5] on mango.

On the other hand, powdery mildew disease caused by *Uncinula necator* is one of the most serious diseases attacking grapevines. In Egypt, *Uncinula necator* was found on different varieties of grapevines causing considerable losses in crop production [6, 7]. In this respect, powdery mildew is widely spread all over the world where temperature is relatively high and moisture occurs as heavy dews rather than as dashing rains [8, 9]. Many substances had been recommended by several workers as an active method for controlling powdery mildew [10, 12]. Among these materials; potassium dihydrogen phosphate was great effective substance in controlling this disease. Some substances contain relatively high amounts of certain chemical compounds which showed inhibitory effects to various fungi as reviewed by Zedan [13] and Zedan *et al.* [14].

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Therefore, the aim of the two experiments was improving the yield and fruit quality of Thompson seedless grape grown under sandy soil conditions through foliar sprays of potassium dihydrogen phosphate (KH_2PO_4) at different concentrations and periods. Also, study some factors affecting spore germination of the causal pathogen of grape powdery mildew, efficacy of some potassium dihydrogen phosphate concentrations compared with fungicide on both disease incidence and disease severity to control the disease under Egyptian conditions.

MATERIALS AND METHODS

This investigation was carried out during two successive seasons (2005 and 2006) and included two experiments:-

First experiment (field exp.): This experiment was done to investigate the effect of spraying potassium dihydrogen phosphate (KH_2PO_4) on leaf mineral content, yield and fruit quality of 6 years old Thompson seedless grape grown on sandy soil at a private vineyard located in Wadi El-Natron, El-Behera Governorate, Egypt.

The results of soil analysis indicated that pH ranged between 7.7 and 7.9, E.C. between 0.82 and 0.76 dsmG[1], $CaCO_3$ ranged between 5.1 and 6.3% and the organic matter between 0.53 and 0.54%. The soil texture was sandy.

For this investigation, 63 vines of almost similar vigor were selected and divided into 7 treatments in three replicates (three vines for each) and arranged in randomized complete block design as the following:

- Control (water spray),
- KH_2PO_4 at 1% every 10 days,
- KH_2PO_4 at 1% every 20 days,
- KH_2PO_4 at 1% every 30 days,
- KH_2PO_4 at 1.5% every 10 days,
- KH_2PO_4 at 1.5% every 20 days,
- KH_2PO_4 at 1.5% every 30 days.

All vines received the normal orchard managements usually practiced in the commercial vineyards located in this area. The application of KH_2PO_4 was carried out as foliar sprays beginning from April to end of July in both seasons.

The following parameters were determined:

- At harvest, number of clusters/vine and yield (kg/vine) were estimated.
- Six clusters were randomly picked from each vine to determine cluster weight (g), fruit weight (g), fruit dimensions (mm), total soluble solids (TSS%) and total acid content (expressed as gm tartaric acid/100 gm juice).
- Leaf mineral contents (total N, P and K %) were determined in petioles from mature leaves (5-7th leaves from shoot top) opposite to basal clusters [15] according to the methods described in Wilde *et al.* [16].

The data were subjected to analysis of variance and Duncan's multiple range test was used to differentiate means [17].

Second experiment (pathological trails):

1 - Laboratory trials

Identification of the causal organism: Fresh leaf specimens representing of Thompson seedless grape showing powdery mildew symptoms were collected from Behera Governorate during 2005 and 2006 seasons. The identification of the causal organism was determined according to Hammouda [18].

Pathogenicity test: Pathogenicity test was carried out in pots (40 cm in diameter) under green house conditions. Disease assessment was calculated as percentages of infected plants after 15 and 30 days of inoculation and reaction to fungal infection was also determined using the method suggested by Barratt and Horsfall [19].

Effect of KH_2PO_4 on spore germination and germ tube length: Percentage of germinated spores was estimated according to El-Naggar [11] as the following formula:

$$\text{Germination \%} = \frac{\text{No. of germinated spores}}{\text{Total No. of spores}} \times 100$$

Whereas germ tube length of germinated spore was measured using slide micrometer 2 mm long (2000 µ).

Effect of KH_2PO_4 on incidence and severity of powdery mildew on detached leaves: Discs (2 cm diameter) of healthy detached leaves of Thompson seedless on distilled water in Petri-dishes were used in this study. Inoculation with powdery mildew conidia on the leaf discs was carried out according to the Lab. technique described by Nagy [20] percentage of infected discs as well as disease severity were determined after 7 days from spraying, according to the methods described by Townsend and Heuberger [21].

2 - Green house trial (*In vivo*)

Effect of foliar spraying with KH_2PO_4 on incidence and severity of powdery mildew: One year old Thompson seedless grape was used in this study. The incidence was determined as the percentage of infected leaves after 7 days from the last spray, whereas, disease severity was determined according to the scale reported by Townsend and Heuberger [21] as follows:

0 = Leaves completely healthy,
1 = 1-2 spots per leaf,
2 = 3-5 spots per leaf,
3 = 6-10 spots per leaf,
4 = Up to 25 percent of the leaf area affected,
5 = Up to 50 percent of the leaf area affected,
6 = Up to 75 percent of the leaf area affected,
7 = More than 75 percent of the leaf area affected.

The percentage of disease severity (D.S.) for each particular treatment was calculated using the following formula:

$$D.S. = \frac{\text{Sum of } (n \times v)}{\text{Total No. of leaves observed in Sample} \times \text{Max. grading}} \times 100$$

Where: n = number of infected leaves in each category. v = numerical value of each category.

Data in these experiments were statistically analyzed using factorial design suggested by Snedecor and Cochran [22]. Least Significant Difference (LSD) at 5% probability was used to compare between treatment averages [23].

RESULTS AND DISCUSSION

First experiment (field exp.):

Leaf mineral content: Results in Table 1 showed the effect of potassium dihydrogen phosphate treatments on N, P and K content in Thompson seedless grape leaves. Generally, all KH_2PO_4 treatments increased N, P and K content in the leaves comparing with the control. This was true in the two seasons of the study. These results are in agreement with those obtained by Abd El-Migeed *et al.* [2] on orange, Eliwa *et al.* [24] on persimmon.

Yield and fruit quality

Yield: Results in Table 2 clearly showed that in the first season, all treatments gave more or less the same values of number of cluster/vine and no differences were detected than the control. While in the second season, treatments 4 and 5 reduced this parameter significantly compared to the other treatments including the control. The average of two seasons showed that the highest value was obtained by spraying KH_2PO_4 at 1.5% every 20 days followed by 1% every 10 days.

Table 1: Effect of KH_2PO_4 sprays on leaf mineral content of Thompson seedless grapevine during 2005 and 2006 seasons

		Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	Treatments	2005	2006	2005	2006	2005	2006
1	Control	2.07c	2.03c	0.09c	0.09c	1.25c	1.27c
2	KH_2PO_4 at 1% every 10 days	2.19a	2.23ab	0.15a	0.15a	1.58a	1.58a
3	KH_2PO_4 at 1% every 20 days	2.02c	2.25a	0.13ab	0.11bc	1.51b	1.50b
4	KH_2PO_4 at 1% every 30 days	2.09b	2.18b	0.15a	0.11bc	1.49b	1.49b
5	KH_2PO_4 at 1.5% every 10 days	2.17a	2.19ab	0.11bc	0.11bc	1.50b	1.51a
6	KH_2PO_4 at 1.5% every 20 days	2.20a	2.23ab	0.15a	0.15a	1.58a	1.58a
7	KH_2PO_4 at 1.5% every 30 days	2.16a	2.18b	0.12b	0.12b	1.49b	1.48b
	Significance at 5% level	S	S	S	S	S	S

Table 2: Effect of KH_2PO_4 sprays on number of clusters/vine, cluster weight and yield weight/vine of Thompson seedless grapevine during 2005 and 2006 seasons

		No. of clusters/vine			Cluster weight (g)			Yield wt./vine (kg)			Increase over
	Treatments	2005	2006	Average	2005	2006	Average	2005	2006	Average	control (%)
1	Control	15.3ab	17.6a	16.5	344g	372e	358	5.2c	6.5c	5.9	---
2	KH_2PO_4 at 1% every 10 days	16.6a	18.0a	17.3	576c	469c	522	9.6ab	8.4ab	9.0	52.0
3	KH_2PO_4 at 1% every 20 days	16.3ab	17.3a	16.8	464f	521a	493	7.5bc	9.0a	8.3	40.0
4	KH_2PO_4 at 1% every 30 days	15.6ab	15.3b	15.5	545d	529a	492	8.5ab	8.1abc	8.3	40.0
5	KH_2PO_4 at 1.5% every 10 days	14.0b	15.0b	14.5	673a	487b	580	9.4ab	7.3bc	8.3	41.0
6	KH_2PO_4 at 1.5% every 20 days	16.3ab	19.3a	17.8	637b	490b	564	10.4a	9.4d	9.9	67.0
7	KH_2PO_4 at 1.5% every 30 days	15.6ab	17.6a	16.6	522e	456d	489	8.1ab	8.0abc	8.1	36.0
	Significance at 5% level	S	S	---	S	S	---	S	S	---	---

Means having the same letters within a column are not significantly different at 5% level

Table 3: Effect of KH_2PO_4 sprays on physical and chemical properties of Thompson seedless grapevine during 2005 and 2006 seasons

		Berry length (mm)		Berry diameter (mm)		Berry weight (g)		TSS (%)		Acidity (%)	
Treatments		2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
1	Control	15.8b	16.2b	12.8b	13.1b	1.90b	1.88d	16.2c	16.3c	0.54a	0.57a
2	KH_2PO_4 at 1% every 10 days	18.0a	18.0a	14.3ab	14.7a	2.45ab	2.37b	18.2ab	18.6a	0.48ab	0.46b
3	KH_2PO_4 at 1% every 20 days	16.7ab	17.9a	13.8ab	14.1ab	2.15ab	2.30bc	17.6ab	17.4abc	0.52ab	0.49b
4	KH_2PO_4 at 1% every 30 days	17.7a	17.7ab	13.4ab	13.6b	2.28ab	2.34b	17.4ab	17.5abc	0.52ab	0.51b
5	KH_2PO_4 at 1.5% every 10 days	17.6a	17.6ab	14.0ab	13.8ab	2.37ab	2.37b	17.6ab	17.6abc	0.50ab	0.51b
6	KH_2PO_4 at 1.5% every 20 days	18.0a	18.0a	14.7a	14.8a	2.59a	2.59a	18.4a	18.2ab	0.46b	0.46b
7	KH_2PO_4 at 1.5% every 30 days	16.6ab	16.5ab	13.3ab	13.2b	2.00ab	2.21c	17.3b	17.2bc	0.51ab	0.50b
	Significance at 5% level	S	S	S	S	S	S	S	S	S	S

Means having the same letters within a column are not significantly different at 5% level

Table 4: Pathogenicity test (infected plants %) on Thompson seedless grape with *Uncinula necator* spores under green house conditions

	Infected plants (%)	
Treatments	15 days after inoculation	30 days after inoculation
Inoculated	35.3	57.5
Control	0.0	0.0
Disease reaction	High susceptible	

As for cluster weight, treatments significantly increased this parameter compared to the control in both studied seasons, where the highest cluster weight was obtained by treatment 5 in the first season, while in the second season treatment 4 followed by treatment 3 gave the best results. However, the average of the two seasons indicted that spraying KH_2PO_4 at 1.5% every 10 days followed by 1.5% every 20 days gave the heaviest cluster.

Regarding yield weight/vine, results revealed that all treatments were effective in increasing yield per vine compared to the control. The highest yield was obtained when KH_2PO_4 was sprayed at 1.50 every 20 days, while the lowest value was obtained by the control. This was true in both seasons of the study and the average of the two seasons. However, the increment in yield caused by potassium dihydrogen phosphate treatments ranged between 36-67% over the control.

The increment in yield weight obtained by KH_2PO_4 sprays could be explained by the improving effect of such treatments on nutritional status of the vines specially the relatively higher leaf NPK% obtained by these treatments which certainly reflected on increasing cluster weight, berry weight and finally yield per vine. These results are supported by Ashour [25] on Anna apple, Abd El-Migeed *et al.* [2] on Hamlin orange, Eliwa *et al.* [24] on Costata persimmon and Elmogy *et al.* [26] on Thompson seedless grapevine, who found that spraying potassium and phosphate increased yield compared to the control plants.

Berries quality: Physical and chemical properties of Thompson seedless grape berries are shown in Table 3.

Physical properties: As for berry length, results indicated that potassium dihydrogen phosphate at any concentration produced longer berries compared with the control. However, spraying KH_2PO_4 at 1% every 10 days or 1.5% every 20 days lengthened berries compared with the other treatments. Similar results were obtained among berry diameter and berry weight, since spraying KH_2PO_4 at 1% every 10 days or 1.5% every 20 days gave the maximum values in both studied seasons and the average of the two seasons.

The above results are in line with those obtained by Elmogy *et al.* [26] who found that potassium spray increased both weight and berry dimensions of Thompson seedless grape. Also the results are in harmony with those finding by Ashour [25] on apple, Abd El-Migeed *et al.* [2] on Hamlin orange.

Chemical properties: As for total soluble solids in berry juice, all treatments significantly increased this parameter compared to the control especially in the first season. The highest percentage was obtained by spraying 1% KH_2PO_4 every 10 days and 1.5% every 20 days. Concerning acidity percentage in berry juice, it was reduced by KH_2PO_4 treatments comparing with the control in both studied seasons.

The obtained results are in line with those reported by Eliwa *et al.* [24] on Costata persimmon, Saleh and Abd El-Monem [5] on Fagri Kalan mango and Elmogy *et al.* [26] on Thompson seedless grape.

From the abovementioned results, it could be concluded that using potassium dihydrogen phosphate as a foliar sprays had a positive effect on leaf mineral content, yield weight and fruit quality of Thompson seedless grapevines specially when sprayed at 1%

Table 5: Effect of different KH_2PO_4 conentrations on spore germination of *Uncinula necator in vitro*, 24 h after inoculation at 25°C and 100% RH

	Germination (%) at KH_2PO_4 conc. (%)					Reduction * (relative to the control)				
Treatments	0.5	1.0	1.5	2.0	2.5	0.5	1.0	1.5	2.0	2.5
Commercial (75%)	45.00	36.30	31.90	17.40	0.00	35.50	48.40	54.30	75.10	100.00
Pure (100%)	35.30	32.50	22.60	9.60	0.00	49.40	53.40	67.60	86.20	100.00
Afugan	16.20	12.00	0.00	0.00	0.00	76.80	82.80	100.00	100.00	100.00
Control (water only)	69.80	69.80	69.80	69.80	69.80	0.00	0.00	0.00	0.00	0.00
LSD at 5% level	5.70	3.95	3.22	2.90	----	5.35	6.13	8.24	4.11	----

* Reduction = Control-treatment×100, Control

Table 6: Effect of KH_2PO_4 concentrations on germ tube length of *Uncinula necator in vitro*, 24 h after inoculation at 25°C and 100% RH

	Germ tube length (µ) at KH_2PO_4 conc. (%)					Reduction (relative to the control)				
Treatments	0.5	1.0	1.5	2.0	2.5	0.5	1.0	1.5	2.0	2.5
Commercial (75%)	32.70	31.80	29.50	22.90	0.00	17.20	19.50	25.30	42.00	100.00
Pure (100%)	28.60	24.00	23.30	19.80	0.00	27.60	39.20	41.00	49.90	100.00
Afugan	12.50	7.60	0.00	0.00	0.00	68.40	80.80	100.00	100.00	100.00
Control (water only)	39.50	39.50	39.50	39.50	39.50	0.00	0.00	0.00	0.00	0.00
LSD at 5% level	3.75	2.65	3.77	2.60	---	3.33	2.40	2.96	3.80	---

Table 7: Effect of spaying with KH_2PO_4 at 2.5% on powdery mildew incidence and severity on detached Thompson seedless grape leaves, artificially inoculated with *Uncinula necator*, 24 h after or before spraying

	(%) Disease incidence		(%) Disease severity	
Treatments	24 h before inoculation	24 h after inoculation	24 h before inoculation	24 h after inoculation
Commercial (75%)	43.10	47.30	32.00	39.60
Pure (100%)	16.70	25.30	13.20	17.50
Afugan	12.30	13.20	10.50	12.60
Control (water only)	66.50	64.00	55.20	63.50
LSD at 5% level	3.43	4.14	2.80	2.41

concentration every 10 days or 1.5% every 20 days from the beginning of April till end of July, which considered the promising treatments under such conditions.

Second experiment (pathological trails)

Identification of the causal organism: Identification trials were carried out according to the conidial stage characteristics [27].

Pathogenicity test: Data in Table 4 indicated that the percentage of infected plants was increased by aging of plants from 15 to 30 days after inoculation. In this respect, Czerniawska *et al.* [28] reported that Thompson seedless grapevine was highly susceptible to powdery mildew disease.

Effect of KH_2PO_4 on spore germination and germ tube of *Uncinula necator*.: Data obtained in Table 5 and 6 show that all tested concentrations of potassium dihydrogen phosphate (KH_2PO_4)were effective in decreasing spore germination percentage and germ tubes length especially under 2.5% compared with the control treatment (water only). However, the commercial and pure salt as well as Afugan fungicide inhibited spore germination and germ tubes length when applied at high concentration (25 g l^{-1}. water).

These results are in agreement with those obtained by Steinhauer and Besser [29]; Seddon and Schmitt [30] and Nair and Arora [31].

The superiority of KH_2PO_4 may be due to: (1) Changing in physio-chemical properties of the salt such as viscosity, pH, acidity …etc. due to the appearance of new compounds and disappearance of the others by heat effectiveness, (2) The high release of the antifungal toxic compounds by heat, (3) High temperature might help anther toxic substance of antifungal effect.

Effect of KH_2PO_4 on incidence and severity of powdery mildew on detached leaves: Spraying Thompson seedless grape detached leaves with KH_2PO_4 at 2.5%, 24 h. before (preventive treatment) or after (curative treatment) artificial inoculation with conidiospores of *Uncinula necator* resulted in significant reduction regarding percentages of disease incidence and severity of powdery mildew comparing with water spray (Table 7).

Table 8: Effect of foliar spraying with KH_2PO_4 on disease and severity of powdery mildew Thompson seedless grape plants, 7 days after artificial inoculation with *Uncinula necator* spores, under green house conditions

Treatments*	Disease** incidence (%)	Reduction*** (%)	Disease severity (%)	Reduction (%)
Commercial (75%)	31.90	60.90	37.20	50.10
Pure (100%)	24.10	70.50	28.80	61.30
Afugan	19.50	76.10	25.70	65.50
Control (water only)	81.60	---	74.50	---
LSD at 5% level	3.55	2.45	5.21	1.86

*Plants were sprayed weekly for 4 weeks with KH_2PO_4 salt, ** Disease incidence and severity were determined 7 days after the last fourth spray treatment, *** Reduction relative to the control (spraying with water only)

Applying potassium dihydrogen phosphate, 24 h. before spores inoculation gave the highest significant reduction concerning the percentage of disease incidence and disease severity. Contrarily, commercial KH_2PO_4 was the least effective treatment on disease incidence and severity. These results are in full accordance with those obtained by Singh and Prithiviraj [32]; Abo-Zied [33] and Sallam, Minaas [34].

Effect of spraying with KH_2PO_4 on powdery mildew disease parameter: Spraying with potassium dihydrogen phosphate significantly decreased powdery mildew infection parameter (incidence and severity) on Thompson seedless grape comparing with water spray only (Table 8). Pure KH_2PO_4 salt (100%) was more effective than those of commercial one (75%) in decreasing disease infection parameters. Percentages of reductions rather than the control for both disease incidence and severity ranged between 70.5 and 61.3%, respectively, in case of pure (100%), while they were 60.9 and 50.1% respectively, with the commercial (75%). On the other hand, reduction percentages in incidence and severity powdery mildew occurred with Afugan fungicide were 76.1 and 65.5% [8, 34-37].

According to the above results obtained from both experiments, it is clear that the efficiency of using potassium dihydrogen phosphate under field conditions was achieved when sprayed at 1.0% every 10 days or 1.5% every 20 days, while it was 2.5% under the pathological trails conditions, it was noticed with 2.5%. So, we suggest more studies under field conditions using higher concentrations of potassium dihydrogen phosphate than 1.5% with different application periods.

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Response of Banana Plants to Soil and Foliar Applications of Magnesium

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Abstract: The effect of soil magnesium fertilization either as sulphate or chelate form with or without $MgSO_4$ foliar sprays was studied on vegetative growth, mineral and leaf chlorophyll content, yield and fruit quality of Grand naine banana plants grown under clay loam soil conditions. Results indicated that Mg fertilization treatments showed a positive effect on vegetative growth parameters, N, Mg, chlorophyll a and b content in the leaves; also improved yield as bunch weight and fruit properties comparing with the control. In this respect, the chelate form was more pronounced than the sulphate form. In addition, treatments included $MgSO_4$ as foliar sprays were more effective than those without it. So, fertilizing Grand naine banana plants with 100 g Mg chelate + foliar sprays of 2% $MgSO_4$ seems to be the promising treatment under this experiment conditions.

Key words: Grand naine banana plants % magnesium fertilization % sulphate % chelate % $MgSO_4$ foliar sprays % leaf minerals % chlorophyll % yield % fruit quality

INTRODUCTION

Banana is considered as one of the most popular and favorite fruits for child and adult. Increasing banana yield, consequently the final profit is the main target for banana producers. They can get their goal through the good orchard management especially fertilization program. In this respect, banana needs a large amounts of nitrogen and potassium fertilization, which depressed magnesium and reduced the ability of trees to Mg uptake [1, 2]. In addition, many symptoms of Mg deficiency have been recently noticed on trees, that received heavy doses of potassium [3, 4]. In this concern, magnesium is a major element and essential on chlorophyll molecule structure, introduce as a co-factor with most enzymes related to active phosphorylation process, also acts as a bridge between pyrophosphate structures of ATP or ADP, the enzyme molecule and stabilizes the ribosome particles in the configuration for protein synthesis [5]. On the other hand, the effect of magnesium on productivity and fruit quality of fruit trees has been documented in Egypt by many investigators on oranges, pear and banana plants, [6-10]. The aim of this study is to investigate the effect of soil magnesium fertilization either as sulphate or chelate form with or without $MgSO_4$ foliar sprays on vegetative growth, mineral and leaf chlorophyll content, yield and fruit quality of Grand naine banana plants grown under clay loam soil conditions.

MATERIALS AND METHODS

This investigation was carried out during 2003-04 and 2004-05 seasons on second and third ratoon Grand naine banana plants grown on clay loam soil in a private plantation at Kfr El-Ziat district, Gharbia Governorate Egypt. Soil physical and chemical analysis are shown in Table 1.

Plants were spaced at 3.5x3.5 meters and three suckers were selected per each hole. Plants under investigation were treated with 100 g of magnesium fertilizer as soil application per each plant either at sulphate (9.8% Mg) or chelate (12.5% Mg EDTA) form with or without foliar sprays of 2% $MgSO_4$. Soil application of magnesium fertilizers was added once at mid May of each season, while the foliar sprays of $MgSO_4$ were applied twice at mid June and mid August of each season. So, this investigation included five treatments as follows:

- Control.
- 100 g of Mg sulphate as soil application.
- 100 g of Mg chelate as soil application.
- 100 g of Mg sulphate as soil application + 2% $MgSO_4$ as foliar sprays.
- 100 g of Mg chelate soil application + 2% $MgSO_4$ as foliar sprays.

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Table 1: Soil physical and chemical analysis

Sand		Silt ----------			Clay		
Course 2000-200 µ%		Fine 200-20 µ%		20-2 µ%	‹2 µ%		Soil texture
8.25		21.30		19.70	50.75		Clay loam
pH (1:2.5)	Ec dsmG^{1} (1:5)	$CaCO_3$%	OM%	N%		ex. P%	ex. K%
8.23	0.46	2.33	1.88	0.40		0.20	0.42
Soluble cations and anions (meq LG^{1})							
Na^+	K^+	Mg^{++}	Ca^{++}	CO_3	HCO_3	Cl	SO_4^-
1.58	0.53	0.68	1.51	----	0.39	3.52	0.39

Each treatment was replicated four times with three plants per each replicate and the randomized complete block design was arranged. The other fertilizing program was the same for all treatments, where each plant received 500 g N/year as ammonium sulphate (20.5% N), 250 g calcium super phosphate (15.5% P_2O_5)/year and 1200 g potassium sulphate (48-52% K_2O)/year. The other cultural practices were the same for all plants.

At shooting stage, leaf sample was taken from the middle of the third leaf from the top of each plant [11], washed with tap water then with distilled water and dried at 70°C till constant weight and finally ground and digested to determine total nitrogen, phosphorus, potassium, calcium and magnesium percentages as the methods described in A.O.A.C. [12]. Chlorophyll a and b were determined according to the method described by Bruinsma [13]. Also the following parameters were determiend for each plant.

- Length and girth of pseudostem.
- Number of green leaves per plant.
- Third leaf area.

At harvest stage, yield as bunch weight (kg) was estimated; also hand and finger weight (g), length and diameter of the finger (cm) were measured. Number of hands and fingers per bunch were calculated for each plant.

After artificial ripening, finger samples were used to determine total soluble solids percentage using hand refractometer. Acidity was measured as g malic acid/100 g pulp, also total sugars percentage was determined according to the methods described in A.O.A.C. [12]. Vitamin C content in the fruit was estimated and expressed as mg of ascorbic acid/ 100 g pulp according to Freed [14].

The data were subjected to analysis of variance and the method of Duncan's was used to differentiate means [15].

RESULTS AND DISCUSSION

Vegetative growth: Results in Table 2 showed that all treatments significantly increased length, girth of pseudostem, number of green leaves and third leaf area comparing with the control. On the other hand, it is noticed that the chelate form of Mg fertilization had a positive effect on vegetative growth parameters compared with the sulphate form. Moreover, treatments included foliar sprays of $MgSO_4$ had a significant effect with respect to pseudostem length and third leaf area compared with the same treatments without foliar sprays. However, foliar sprays seemed to be ineffective on pseudostem girth and number of green leaves. The higher values of length, girth of pseudostem, number of green leaves and third leaf area were obtained when the plants fertilized with 100 g Mg chelate + 2% $MgSO_4$ foliar sprays, while the lower values of these parameters were recorded with the control plants.

The positive effect of magnesium fertilization on vegetative growth parameters are in harmony with those obtained by Abd El-Kader *et al.* [16], Turner and Barker [17] and Guillier [18] on different banana varieties.

Mineral and chlorophyll content in the leaves: Data in Table 3 showed mineral and chlorophyll content in the leaves as affected by Mg fertilization forms with or without foliar sprays of $MgSO_4$. As for nitrogen, all treatments significantly increased N leaf content than the control. However, it is clear that the chelate form of Mg fertilization recorded higher N values compared with the

Table 2: Vegetative growth of grand naine banana plants as affected by soil and foliar sprays of magnesium

	Pseudostem							
	Length (cm)		Girth (cm)		No. of green leaves		Third leaf area (m^2)	
Treatments	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05
Control	269e	272e	80c	82c	11.7c	12.0d	1.75e	1.78e
100 g soil $MgSO_4$	285d	290d	92b	92b	13.1bc	13.3c	2.05d	2.08d
100 g soil Mg chelate	297b	300b	98a	98a	13.8a	13.8b	2.13c	2.16c
100 g soil $MgSO_4$+2% foliar $MgSO_4$	292c	295c	94b	94b	13.5ab	13.7b	2.20b	2.24b
100 g soil Mg chelate+2% foliar Mg SO_4	318a	321a	98a	98a	14.1ab	14.4a	2.40a	2.43a
Significance at 5% level	S	S	S	S	S	S	S	S

Means having the same letter(s) within a column are not significantly different at 5% level

Table 3: Minerals and chlorophyll content in Grand naine banana leaves as affected by soil and foliar sprays of magnesium

											Chlorophyll mg gG^{1} fresh weight			
	N (%)		P (%)		K (%)		Ca (%)		Mg (%)		A		B	
Treatments	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05
Control	2.58e	2.63d	0.19	0.20	3.25a	3.38a	0.28	0.25	0.33b	0.32c	0.68d	0.70d	0.19c	0.19d
100 g soil $MgSO_4$	2.85d	2.83c	0.22	0.21	2.81c	2.84c	0.25	0.22	0.39a	0.39b	0.88c	0.91c	0.23bc	0.23cd
100 g soil Mg chelate	3.10b	3.07b	0.21	0.23	2.92b	2.93b	0.25	0.23	0.40a	0.41ab	0.98b	0.97b	0.28ab	0.31ab
100 g soil $MgSO_4$ + 2% foliar $MgSO_4$	2.97c	2.94bc	0.21	0.22	2.94b	2.95b	0.24	0.21	0.41a	0.42ab	0.87c	0.90c	0.24bc	0.26bc
100 g soil Mg chelate + 2% foliar Mg SO_4	3.42a	3.44a	0.23	0.23	2.97b	2.98b	0.23	0.22	0.45a	0.46a	1.16a	1.26a	0.31a	0.33a
Significance at 5% level	S	S	NS	NS	S	S	NS	NS	S	S	S	S	S	S

Means having the same letter(s) within a column are not significantly different at 5% level

sulphate form either with or without foliar sprays. Also, it is noticed that treatments included foliar sprays significantly raised N% compared with the same treatment without it, especially with the chelate form. Moreover, treatment included 100 g chelate Mg+2% $MgSO_4$ as foliar sprays gave the highest value in both studied seasons.

Phosphorus percentage in the leaf was not significantly affected by treatments, although a slight increase was detected by all treatments than the control in the two seasons.

Regarding potassium content in the leaf, results indicated that the control plants recorded the highest significant K% in the leaf than all other treatments. On the other hand, the Mg chelate form increased K value than the sulphate form but this increment was significant only without adding foliar sprays. Similarly, foliar sprays of $MgSO_4$ enhanced K% in the leaves than without spray but the significance was observed when the plants fertilized with sulphate form only.

Calcium content in the leaves was not affected by treatments. However, the control treatment gave the highest Ca value in the leaf compared with the other treatments.

Concerning magnesium content in the leaves, all treatments significantly increased Mg value than the control. However, a gradual increase in Mg content was observed among all Mg treatments, but this increment lacked significance in the first season, while in the second one, Mg chelate fertilizer recorded the highest Mg content in the leaves.

As for chlorophyll a and b content in the leaves, there was a significant effect for treatments on these parameters, since a gradual increment was observed among the treatments in compared with the control which recorded the lowest value, while the highest value was obtained when the plants fertilized with 100 g Mg chelate + 2% $MgSO_4$ foliar sprays. On the other hand, the chelate form show a significant increase compared with the sulphate one. Moreover, using $MgSO_4$ as foliar sprays did not show any effect with respect to chlorophyll b, while it significantly increased chlorophyll a when sprayed with chelate form.

From the previous results, it is clear that Mg fertilization treatments showed a positive effect on N, Mg, chlorophyll a and b comparing with the control. While, it had a negative effect on K% and had no effect on P and Ca content in the leaves. In this respect, the chelate form

Table 4: Yield and fruit quality of Grand naine banana plants as affected by soil and foliar sprays of magnesium

	Bunch wt. (kg)		No. hands/bunch		No. fingers/bunch		Hand wt. (kg)		Finger length (cm)		Finger diameter (cm)		Finger wt. (g)	
Treatments	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05
Control	24.0d	24.7e	9.3d	9.7d	161d	166d	2.60	2.57b	15.7e	15.4e	2.60d	2.77d	148b	158d
100 g soil $MgSO_4$	29.7c	30.7d	11.3c	12.0c	186c	188c	2.60	2.57b	17.4d	17.6d	3.30c	3.37c	159a	162a
100 g soil Mg chelate	34.0b	35.0b	13.0ab	13.0b	210b	212b	2.60	2.70a	19.3b	19.6b	3.63b	3.67b	162a	164a
100 g soil $MgSO_4$ + 2% foliar $MgSO_4$	31.0c	32.3c	12.3bc	13.0b	188c	192c	2.53	2.50b	18.7c	19.2c	3.57b	3.67b	165a	168a
100 g soil Mg chelate + 2% foliar Mg SO_4	35.7a	36.7a	13.7a	14.0a	221a	225a	2.63	2.60ab	20.9a	21.8a	4.13a	4.2a	161a	163a
Significance at 5% level	S	S	S	S	S	S	NS	S	S	S	S	S	S	S

Means having the same letter(s) within a column are not significantly different at 5% level

Table 5: Chemical properties of Grand naine banana fruits as affected by soil and foliar sprays of magnesium

	TSS (%)		Total sugars (%)		Acidity (%)		Ascorbic acid (mg/100 g)	
Treatments	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05
Control	15.3e	15.8e	15.1e	15.0e	0.29	0.31	15.2c	15.1e
100 g soil $MgSO_4$	17.0d	17.2d	16.7d	17.0d	0.31	0.32	16.4b	16.2d
100 g soil Mg chelate	19.0c	19.9c	18.4c	18.6c	0.32	0.32	16.7b	16.7b
100 g soil $MgSO_4$ + 2% foliar $MgSO_4$	20.5b	21.2b	19.9b	20.2b	0.33	0.34	16.5b	16.4c
100 g soil Mg chelate + 2% foliar Mg SO_4	22.2a	22.9a	21.5a	22.0a	0.35	0.35	17.5a	17.4a
Significance at 5% level	S	S	S	S	NS	NS	S	S

Means having the same letter(s) within a column are not significantly different at 5% level

was more pronounced than the sulphate form; also treatments included Mg sprays were more effective than those without it. These results are agreed with those recorded by Abou Aziz *et al.* [9] and Abd El-Kader *et al.* [16] on banana, El-Safty and Rabeii [8], Maksoud *et al.* [19] and Abd El-Moniem *et al.* [20] on oranges, who mentioned that an increase in N, Mg and chlorophyll, while a reduction in K content in the leaves was observed due to magnesium applications.

Yield and fruit quality: Results in Table 4 indicated that bunch weight was affected by different treatments. However, all treatments significantly increased bunch weight in compared with the control in both seasons. In this respect, the highest significant weight was recorded when the plants fertilized with 100 g Mg at chelate form +2% $MgSO_4$ foliar sprays. Moreover, treatments included the chelate Mg form significantly increased bunch weight than those fertilized with Mg sulphate form either with foliar sprays or not. On the other hand, treatments included $MgSO_4$ as foliar sprays raised bunch weight especially with Mg chelate treatment. Similar results were obtained with respect to number of hands and fingers/bunch. Hand weight was affected in the second season only, since Mg chelate treatments improved hand weight than Mg sulphate treatments, while spraying $MgSO_4$ had no effect on this parameter. In this concern, fertilizing plants with 100 g Mg chelate solely recorded the heaviest hand. This was true in the second season only. Finger weight was affected by treatments, since all Mg fertilization treatments significantly increased finger weight compared with the untreated plants (control). No significant differences were detected between Mg fertilization treatments. This was true in both studied seasons. Regarding length and diameter of the finger, results indicated that, both of the two parameters toke the same trend. Since, all treatments significantly increased length and diameter of finger than the control. Also treatments included Mg chelate form and foliar sprays improved these parameters than those included sulphate form without foliar sprays.

Data in Table 5 indicated that TSS percentage and total sugars in banana fruit followed the same trend, since all treatments significantly increased these parameters comparing with the control plants. On the other hand, chelate treatments increased TSS and Total sugars in comparing with the sulphate treatments, also foliar sprays treatments increased the same parameters than without foliar sprays. Moreover, it is clear that treatment included Mg chelate + $MgSO_4$ foliar sprays gave the highest value for both TSS and total sugars content in the fruit. As for

acidity percentage, treatments had no effect on this parameter although there is an increasing trend through the treatments in compared with the control. Ascorbic acid content in the fruits was significantly increased by all treatments than the control and the highest significant value was obtained when the plants fertilized with Mg chelate and $MgSO_4$ foliar sprays.

The positive effect of magnesium fertilization may be due to the important physiological role of magnesium on chlorophyll molecule structure, enzymes activity and protein synthesis [5] that reflected on increasing growth parameters and consequently improved yield and fruit quality of banana plants.

The previous results are in line with those obtained by Abou Aziz *et al.* [9] and Abd El-Kader *et al.* [16] on banana, El-Safty and Rabeii [8], Maksoud *et al.* [19] and Abd El-Moniem *et al.* [20] on oranges, Attala *et al.* [7] on Le Conte pear and El-Seginy *et al.* [21] on Anna apple.

From the abovementioned results, it could be concluded that soil magnesium fertilization had a positive effect on banana plants with or without Mg spray. However, fertilizing Mg at chelate form was more pronounced than the sulphate one. Moreover, using $MgSO_4$ as foliar sprays was more effective than without it especially when applied with Mg chelate form. So, fertilizing Grand naine banana plants with 100 g Mg chelate as soil application + foliar sprays of 2% $MgSO_4$ seems to be the promising treatment under this experiment conditions.

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Influence of Zn and Mn Levels on Growth and Micronutrient Acquisition of Apple Microculture

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Abstract: Growth and micronutrient acquisition of two apple rootstock (MM106 and M 26) and one cultivar (Galla) in response to different level of Zn (0.8, 1.6, 4.8, or 6.4 mg LG^{1}) and Mn (2.7, 5.4, 16.2 or 21.6 mg LG^{1}) concentration were studied *in vitro*. Microshoot fresh weight and dry weight were increased with increasing Zn level in the medium in all apple varieties. Similar response was shown for leaf number, number of shoot and microshoot length with increase Zn up to 4.8 mg LG^{1} and decreased at higher level. Zn acquisition in all varieties was elevated with increasing Zn level in the medium, while Zn had an antagonistic effect on Mn and Cu concentration at high Zn level (6.4 mg LG^{1}). Antagonistic effect of Zn was observed on Fe concentration on Galla and M26 and a synergistic effect was obtained in MM106 tissues. In Mn experiment, microshoot fresh weight, dry weight and length in all apple varieties increased with increasing Mn level (up to 16.2 mg LG^{1}) in the medium. Meanwhile, leaf number and number of shoots decreased with increasing Mn level in MM 106 and M 26. Increasing Mn level in the medium had a positive effect on Zn uptake in MM 106 and Galla tissues and Zn uptake decreased in M 26. Cu acquisition in Galla was decreased with elevated Mn level in the medium. In Galla and M 26 varieties Mn had a positive effect on Fe concentration.

Key words: Acquisition % apple % micronutrient % microculture

INTRODUCTION

Micronutrients availability and utilization by plants are highly dependent on soil and climatic factors, such as, soil type, aeration, water and mineral interaction [1-3]. Basic soil reaction and $CaCO_3$ content in the soil significantly limit the availability of Zn and Mn. Under these soil conditions, plant genotype that genetically controls the use efficiency of soil nutrients, can play a key role in enhancing Zn and Mn acquisition under unfavorable soil conditions. Moreover, varieties within each genotype may have differential response to low or toxic levels of soil micronutrients [4, 5].

In tissue culture, growth medium was designed to provide the plant tissues with mineral nutrients (macro and micronutrients) that are necessary for *in vitro* and development. Ratios of nutrients in the medium were stated to mimic those found in plant tissues after ashing [6] which represent the actual plant needs of minerals *in vitro*. So using tissue culture techniques have made it possible to estimate plant needs in different growth stages of micronutrients that might taken as a guide in many fertilization programs [7, 8].

Tissue culture has been considered a relevant system for selection of plant tolerance to the imposed treatment in the medium [9]. *In vitro* cultures offer greater control and precise measurement for growth and development of plant tissues [9-11]. The use of tissue culture can help to focus on the physiological and biochemical mechanisms, which help plant tolerance to medium-included stress [10-13]. It has been reported that the whole plant response to stress conditions at the cellular level and those of the tissue culture are similar [11, 12]. In addition, tissue culture been used to assess plant reaction to salinity [9, 13] or drought [14] in many plant species. Although several studies [14-16] have been conducted *in vitro* to

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monitor plant genotypes responses to low, normal and even toxic levels of a certain micronutrient under controlled environment. More research is needed to investigate more genotypes and to study the effect of different levels of Zn and Mn in the medium on growth and acquisition of other micronutrients. This would also help in screening varieties that are resistant to either micronutrient deficiency or toxicity. In the study, growth and micronutrient acquisition of apple rootstocks (MM 106, M 26) and one cultivar (Galla) in response to deferent levels of Zn and Mn were evaluated *in vitro*.

MATERIALS AND METODS

Two apple rootstocks (MM 106 and M 26) and one cultivar (Galla) were established *in vitro* according to Shibli *et al.* [11]. Mature dormant wood (one year) was collected in late winter and cut into section (10 cm long). The basal end of each section was immersed in tap water at room temperature 24±2°C day and 18±2°C night for four weeks until buds broke their dormancy. Buds were excised and surface sterilized in 5.52% sodium hypochlorite and rinsed aseptically with sterile distilled water. Shoot tips (0.5-0.7 mm) were excised and inoculated on MS medium [17] supplemented with 30 g L^{-1} sucrose, 1.0 mg L^{-1} N^6-Benzyl adenine (BA), 0.1 mg L^{-1} gibberellin (GA_3) and 8.0 g L^{-1} Bacto ager. Cultures were transferred to the growth room and maintained under daily regime of 16 h light (photosynthetic photon Flux Density; PPFD = 40-45 µmol m^{-2} s^{-1})/8 h dark and incubated at 22±2°C and subculturing was performed every four weeks.

For Zn experiment, microshoots (1.0 cm) were transferred into a full strength MS medium supplemented with different Zn concentration (0.8, 1.6, 4.8 and 6.4 mg L^{-1}) added from ZnSO4.7H20 (1.6 mg L^{-1} = regular Zn concentration described in MS medium). For Mn experiment, other microshoot were subculture into MS medium supplemented with different Mn level (2.7, 5.4, 16.2 and 21.6 mg L^{-1}) added from MnSO4.4H2O (5.4 mg L^{-1} = regular Mn concentration described in MS medium). The medium was also supplemented with 0.5 mg L^{-1} 6-Benzyladenine, 2.0 mg L^{-1} gibberelline, 0.1 mg L^{-1} Indolebutyric acid (IBA), 30 g L^{-1} sucrose and 8 g L^{-1} Bacto agar and pH was adjusted to 5.7. The cultures were maintained under similar growth room conditions described above. Each treatment consisted of ten replicates arranged in a Complete Randomized Designed. Each experiment was repeated 3 times.

The experiment duration was 4 weeks and at the end of each experiment; data were collected on fresh weight, leaf number, number of proliferated shoots, number of dead leaves and microshoots length. Microshoots were then oven dried at 70°C and dry weight was recorded. Dry microshoots were milled and allowed to pass 1.0 mm sieve size before dry ashing at 55°C. Micronutrient (Zn, Mn, Cu and Fe) were analyzed using an atomic absorption spectrophotometer (Pye Unicam Sp9). Data collected for growth were analyzed statistically according to analysis of variance ANOVA and mean were separated using Least Significant Difference (LSD) test at probability of (0.05). For micronutrient data, the Standard Deviation (SD) between two concentrations of each micronutrient was calculated.

RESULTS AND DISCUSSION

All apple varieties showed some increased in microshoot fresh and dry weight in response to increasing Zn acquisition in the medium (Table 1). Similar results were obtained in tomato and cucumber shoot grown on solution medium supplemented with elevated levels of Zn [4, 15]. Leaf number and number of shoots, however, were highest when Zn concentration was 4.8 mg L^{-1} in the medium. These trends indicated no varietals differences in response to Zn level used in this study (Table 1). Number of dead leaves was decreased in the MM 106 variety when Zn level was lowered below 1.6 mg L^{-1}. Highest number of dead leaves of Galla was at Zn level of 1.6 mg L^{-1}, while with M 26 dead leaves were increased as Zn level increased in the medium. This may suggest that M 26 was the most sensitive to the increased level of Zn. Microshoot length increased in MM 106 and Galla along with the increased Zn level (up to 4.8 mg L^{-1}). However, it was reported that Zn has a role in activation of enzyme responsible of auxin synthesis [18] that intern might be toxic enough to inhibit microshoot elongation. Although M 26 had higher number of dead leaves as Zn increased in the medium, the length of the microshoot was highest.

Increasing Zn concentration in the medium was accompanied with continuous increase in Zn acquisition in an all apple varieties (Table 2). Similar results were reported in tomato and cucumber [4, 11]. However, MM 106 was more efficient in Zn acquisition from the medium at all Zn levels. Similar trend was observed in Mn acquisition in M 106 and Galla as Zn level increased to 4.8 mg L^{-1} (Table 2). Higher levels of Zn (6.4 mg L^{-1}) resulted in lower Mn concentration in the tissues suggesting a negative effect on Mn at the highest Zn level in the medium for all varieties. An obvious

Table 1: Effect of medium Zn concentration on microshoot growth of different apple varieties grown *in vitro*. Medium supplemented with 0.5 mg LG^{1} BAp, 2.0 mg LG^{1} GA_3 and 0.1 mg LG^{1} IBA

Apple variety	Zn conc. (mg LG^{1})	Fresh weight (g)	Dry weight (g)	Leaf number	Number of shoots	Number of dead leaves	Microshoot length (cm)
MM 106	0.8	0.320	0.032	11.60	1.8	3.8	1.86
	1.6[a]	0.322	0.037	18.00	2.2	2.2	1.98
	4.8	0.341	0.066	12.20	1.4	1.4	4.16
	6.4	0.375	0.088	11.60	1.2	1.0	2.16
Galla	0.8	0.237	0.017	6.40	1.0	2.0	1.12
	1.6	0.296	0.033	24.60	1.8	3.2	2.74
	4.8	0.321	0.057	13.40	1.4	2.6	3.20
	6.4	0.374	0.068	9.40	1.2	1.8	2.28
M 26	0.8	0.271	0.035	14.40	1.0	1.8	1.04
	1.6	0.320	0.040	16.21	1.4	2.0	1.48
	4.8	0.327	0.051	14.20	1.0	3.2	2.04
	6.4	0.341	0.054	6.80	1.0	4.2	2.36
LSD		0.019	0.004	0.947	0.167	0.253	0.200

a = Same as the regular Zn level in the MS medium. Means were separated using Least Significant Differences (LSD) test at p = 0.05. Means±standard deviation, n = 3. Approximately 10-12 microshoots were tested for each replicate

Table 2: Effect of medium Zn concentration on micronutrient acquisition in different apple varieties grown *in vitro*. Medium supplemented with 0.5 mg LG^{1} BAp, 2.0 mg LG^{1} GA_3 and 0.1 mg LG^{1} IBA

		Mineral content (µg gG^{1})			
Apple variety	Zn conc. (mg LG^{1})	Zn	Mn	Cu	Fe
MM 106	0.8	240.31(0.9)	110.00(0.0)	46.25(3.7)	31.25(0.0)
	1.6[a]	342.27(0.8)	200.63(1.2)	36.20(5.6)	116.66(0.3)
	4.8	417.29(0.9)	232.46(0.4)	26.11(2.8)	246.51(0.5)
	6.4	594.47(0.3)	228.40(0.8)	15.50(0.8)	250.23(0.3)
Galla	0.8	81.13(0.0)	83.77(1.9)	20.29(6.2)	162.90(2.0)
	1.6	130.70(1.5)	160.00(0.5)	21.25(7.8)	174.10(4.0)
	4.8	391.18(0.0)	178.07(1.1)	35.83(2.4)	241.29(2.0)
	6.4	489.44(3.6)	123.15(1.8)	21.80(4.7)	210.60(2.0)
M 26	0.8	283.16(0.3)	217.18(2.4)	70.00(1.6)	731.25(2.0)
	1.6	326.87(0.8)	216.32(0.5)	68.43(2.6)	565.70(0.0)
	4.8	356.88(0.9)	199.71(1.4)	59.14(4.8)	526.32(0.0)
	6.4	478.23(0.3)	171.70(1.5)	34.41(0.9)	308.85(0.6)

a = Same as the regular Zn level in the MS medium. Means were separated using Least Significant Differences (LSD) test at p = 0.05. Means±standard deviation, n = 3. Approximately 10-12 microshoots were tested for each replicate

antagonistic effect of Zn concentration on Cu acquisition was recorded in MM 106 and M 26, while Cu acquisition declined in Galla and M 26 varieties was similar to that of Zn and Mn. The MM 106, however continued to take up more Fe even at highest Zn concentration in the medium (Table 2).

In Mn experiment, fresh and dry weight of microshoots increased with increasing Mn level in the medium (up to 16.2 mg LG^{1}) and any increase beyond this concentration caused a decrease in both weight (Table 3). Increasing Mn concentration in the medium decreased leaf number of shoots in MM 106 and M 26 varieties but this trend was the opposite in Galla (Table 3). Microshoots length showed some increase with increasing Mn level to 16.2 mg LG^{1} in all varieties then decline at higher Mn levels (Table 3).

Increasing Mn level in the medium resulted in increased level of Zn in tissue by MM 106 and Galla. M 26 also took up more Zn with increasing Mn level until Mn reached 16.2 mg LG^{1} (Table 4), where negative interaction between Zn and Mn was observed [8]. Mn acquisition

Table 3: Effect of medium Mn concentration on microshoot growth of different apple varieties grown *in vitro*. Medium supplemented with 0.5 mg L^{-1} BAp, 2.0 mg L^{-1} GA_3 and 0.1 mg L^{-1} IBA

Apple variety	Zn conc. (mg L^{-1})	Fresh weight (g)	Dry weight (g)	Leaf number	Number of shoots	Number of dead leaves	Microshoot length (cm)
MM 106	2.7	0.327	0.024	18.20	1.60	1.00	3.40
	5.4[a]	0.507	0.056	16.80	1.60	0.60	3.90
	16.2	0.602	0.102	16.00	1.00	0.20	4.30
	21.6	0.543	0.086	9.20	1.00	2.00	1.84
Galla	2.7	0.330	0.059	15.60	2.40	0.80	3.20
	5.4	0.430	0.066	16.40	2.40	0.40	3.34
	16.2	0.469	0.088	18.80	3.00	1.00	4.12
	21.6	0.38	0.082	20.40	1.20	2.00	3.40
M 26	2.7	0.433	0.068	28.00	4.80	1.60	1.60
	5.4	0.498	0.078	26.40	3.40	2.00	2.70
	16.2	0.591	0.154	18.60	2.20	1.00	3.88
	21.6	0.568	0.107	14.80	1.60	1.60	2.62
LSD		0.027	0.001	1.29	0.20	0.22	0.35

a = Same as the regular Zn level in the MS medium. Means were separated using Least Significant Differences (LSD) test at p = 0.05. Means±standard deviation, n = 3. Approximately 10-12 microshoots were tested for each replicate

Table 4: Effect of medium Mn concentration on micronutrient acquisition in different apple varieties grown *in vitro*. Medium supplemented with 0.5 mg L^{-1} BAp, 2.0 mg L^{-1} GA_3 and 0.1 mg L^{-1} IBA

		Mineral content (µg g^{-1})			
Apple variety	Zn conc. (mg L^{-1})	Zn	Mn	Cu	Fe
MM 106	2.7	80.78(0.3)	42.82(1.6)	10.86(0.4)	149.13(0.6)
	5.4[a]	148.66(0.0)	101.40(0.9)	20.00(0.5)	144.77(1.0)
	16.2	239.99(0.0)	116.31(0.2)	15.69(0.7)	100.43(0.8)
	21.6	245.82(0.3)	138.53(0.1)	13.29(01.7)	93.90(2.0)
Galla	2.7	41.00(1.1)	40.90(0.8)	10.13(1.5)	41.25(1.0)
	5.4	184.70(0.4)	105.00(0.7)	10.50(2.0)	97.70(1.4)
	16.2	239.36(0.3)	131.37(1.1)	19.84(2.4)	176.20(0.6)
	21.6	283.78(0.1)	168.88(0.2)	15.33(1.1)	207.14(0.7)
M 26	2.7	126.25(0.3)	50.00(0.3)	11.38(1.2)	83.30(0.7)
	5.4	238.75(0.1)	90.50(0.4)	18.39(1.4)	135.00(1.0)
	16.2	277.75(0.2)	150.75(0.4)	16.37(3.7)	137.1(0.1)
	21.6	110.00(0.6)	165.10(0.1)	8.90(0.1)	148.44(1.0)

a = Same as the regular Zn level in the MS medium. Means were separated using Least Significant Differences (LSD) test at p = 0.05. Means±standard deviation, n = 3. Approximately 10-12 microshoots were tested for each replicate

increased along with increasing along with increasing Mn level in the medium in all apple varieties (Table 4). In MM 106 and M 26, Cu concentration in tissue was the highest for the control treatment and continued to decrease with increasing Mn. This suggests an antagonistic effect of Mn on Cu in these two varieties. Meanwhile, Cu concentration in Galla continued to increased with increasing Mn level (up to 16.2 mg L^{-1}) in the medium (Table 4). Other researcher found a negative interaction between Mn and Fe but not with Cu [19, 20, 22]. Mn is suggested to interfere with Fe transport in annual crop [15] Fe concentration decreased with increasing Mn level in the medium in MM 106 tissues but this trend was the opposite in Galla and M 26 varieties (Table 4).

Antagonistic interaction between Mn and Fe was observed in avocado trees but not in apple tree [15, 21, 22]. This suggests variations among plant species

in response to differential level of Mn and Fe. Our results suggest, furthermore, varietals variations in their response to Mn and Fe levels. This indicates a positive Mn/Fe relationship in Galla and M 26 varieties while an antagonistic on Fe was observed with MM 106. Antagonistic interaction has been reported among micronutrients [23]. However, these results indicate a significant difference among varieties in their response to different level of micronutrient and to the deficient and/or toxic levels of micronutrient.

CONCLUSIONS

Growth and micronutrient acquisition of the three tested apple varieties were influenced by increasing Zn or Mn level in the medium. Generally, most growth parameters and acquisition of some micronutrients in all apple varieties increased with increasing the Zn or Mn level until the regular micronutrient concentration was tripled as they decline at such high level. Also apple varieties varied in their response to the experimental treatments. This might refer to the varietal sensitivity that varies form one variety to another in micronutrient acquisition. Therefore, this can be suggested as a tool to screen varieties for their responses to deficient and/or toxic level of micronutrient and to their interaction.

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Effect of Plant Spacing and Date of Planting on Yield of Two Garlic (*Allium sativum* L.) Cultivars in Sokoto, Nigeria

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Abstract: Two field experiments were carried out during the 1997 and 1998 dry seasons at the Usmanu Danfodiyo University Teaching and Research Fadama Farm Sokoto[13° 01'N Latitude and 5° 15'E Longitude], 350 meters above sea level, in order to study the response of two garlic (*Allium sativum* L.) cultivars to varying plant spacings and planting dates. A factorial combination of three planting dates (Nov. 29, Dec. 13 and Dec. 27), four plant spacings (5, 10, 15 and 20 cm) and two cultivars (ex-Lugu and ex-Kofa) were laid out in a Complete Randomized Block Design and replicated three times. Each gross plot was 1.5 m wide and 2.0 m long (3 m^2) consisting of ten rows, while the net plot was 0.9×1.4 m (1.26 m^2) consisting of six inner row. Data was collected on fresh and cured bulb yield and analysed. Results indicated that close spacing of 10 cm resulted in higher cured bulb yield, while early plantings Nov. 29 and Dec. 13 resulted in higher yield than late planting of Dec. 27. The local cultivar ex-Lugu out yielded the ex-Kofa. It is therefore suggested that garlic be planted early in the season (Nov. 29-Dec. 13) at 10 cm spacing using the local ex-lugu cultivar in Sokoto.

Key words: Plant spacing % date of planting % cultivar and garlic yield

INTRODUCTION

Garlic (*Allium sativum* L.) belongs to the family Alliaceae. Other crops in the family are onion (*A. cepa* L.), leek (*A. ameloprisum* L.), shallot (*A. asacloncum* L.) and chive (*A. schoenoprasum* L.). Garlic is the second most widely used of the cultivated bulb crops after onions. It is an erect annual herb that can reach a height of 75-90 cm and grows during dry and mild winter season [1].

Garlic is believed to have originated in Central Asia (India, Afghanistan, W. China, Russia etc.) and spread to other parts of the world through trade and colonization [2, 3]. According to FAO [4], production of garlic stood at about 10 million tonnes per annum which is only about 10% that of bulb onions. China is the world largest producer followed by South Korea. The world average yield of garlic is about 10 t ha^{-1}, but can go up to 19 t ha^{-1}.

Garlic is rich in sugar, protein, fat, calcium, potassium, phosphorus, sulfur, iodine, fibre and silicon, in addition to vitamins. Its pungent flavor makes it used mainly as a spice, seasoning and flavoring for foodstuff involving both green tops and bulbs. Its medicinal value is also well recognized in the control and treatment of hypertension, worms, germs, bacterial and fungal diseases, diabetes, cancer, ulcer, rheumatism etc. Dehydrated garlic and extracts are fast replacing fresh bulbs for industrial and home usage in the production of drugs, insecticides and explosives [2].

Sokoto is among the leading garlic producing states in Nigeria. Production of the crop dates back several decades in the state. Garlic cultivation covers an area of over 11,277 ha producing about 73,908 metric tonnes per annum [5]. The main producing Local Government areas are Goronyo, Wurno, Gada, Gwadabawa, Rabah and Kware, where the crop is grown under irrigation during the cool dry season (Hamattan) in Nov-March. The traditional husbandry practices have resulted in 3-4 t ha^{-1} yield as against the world average of 10-15 t ha^{-1}. At off-season same quantity of garlic is usually sold at twice or three times the value of onions. It is mostly exported to parts of Africa, Middle East, Asia and Europe through various trade routes. A number of studies in various parts of the world have shown that garlic production can be improved through appropriate cultural practices [6-8]. Unfortunately, there is dearth of information on garlic production in Nigeria, except for some recent work at Samaru on spacing, fertilizer and irrigation requirements [9-11].

It is in view of this background that this study was undertaken with the aim of exploring opportunities to improve the productivity of the crop through choice of

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appropriate plant spacing, planting date and cultivar that maximizes the yield factors.

MATERIALS AND METHODS

Two field experiments were carried out during the 1997 and 1998 dry seasons (November to March) at Usmanu Danfodiyo University, Teaching and Research Fadama Farm Sokoto [13° 01'N Lat. 5° 15'E Long; 350 meters above sea level] in order to study the response of two garlic (*Allium sativum* L.) cultivars to varying levels of plant intra-raw spacings and planting dates.

The treatments consisted of the two cultivars planted at three planting dates at two weeks interval (Nov. 29, Dec. 13, Dec. 29) and four inter-plant spacings (5, 10, 15 and 20 cm). The treatments were factorially laid out in Randomized Complete Block Design replicated three times.

Land preparation were carried out 14-18th November. Each gross plot was 1.5 m wide and 2.0 m long (3 m^2) consisting of ten rows, while the net plot was 0.9×1.4 m (1.26 m^2) consisting of six inner row. The experimental units used were ex-Lugu and ex-Kofa garlic cultivars. While the ex-Lugu is popularly grown in Lugu Village of Wurno Local Government area of Sokoto State whose mature cloves have light pinkish outer skin. The ex-Kofa is a local garlic cultivar popularly grown in Kofa Village in Bebeji Local Government area of Kano State. Its mature cloves have light pinkish covering scale.

Prior to planting, garlic bulbs were split into the individual cloves that were soaked in water over-night. The cloves were planted upright with epical tip exposed at 10 cm inter row spacing according to the treatment structure. The field was irrigated before sowing in order to provide good clove-soil-water contact. Subsequent irrigations were given at 7 days intervals.

Fertilizer was applied at 90 kg N, 22 kg P and 26.4 kg K per hectare in form of Urea (46% N), N.P.K (20-10-10) and single super phosphate (18% P_20_5 ha^{-1}). A basal dose of half the nitrogen rate and full doses of the phosphorus and potassium were applied at planting. The second half dose of the nitrogen was applied at four weeks after sowing.

There was no incidence of either pest or disease in the crop throughout the growth period of experimentation in both seasons except for the nut sedge (*Cyprus rotundus*) that was controlled manually hand picking and hand hoeing.

Bulbs were harvested when the leaves turned yellowish green and had started withering. The harvested bulbs were spread in single layers in an open space for two weeks for curing.

Table 1: Fresh bulb yield (kg ha^{-1}) as affected by plant spacing, planting date and cultivar during 1997 and 1998 dry seasons and combined analysis at Sokoto

	Fresh bulb yield		
Treatments	1997	1998	Combined
Nitrogen (kg ha^{-1})			
0	8131	4416b	6273b
60	7522	8047a	7785a
120	8140	8364a	8243a
180	7811	6658a	7234ab
240	7289	7271a	7280ab
Significance	ns	**	*
L.S.D.	1567	2162	1321
Phosphorus (kg ha^{-1})			
0	7698	6430	7064
22	7429	7376	7403
44	8208	7048	7628
Significance	ns	ns	ns
L.S.D.	1214	1675	1023
Cultivar			
Ex-Lugu	8121	6980	7550
Ex-Kofa	7436	6923	7180
Significance	ns	ns	ns
L.S.D.	991	1367	835
Interaction			
N*P	ns	ns	ns
N*C	ns	ns	ns
P*C	ns	ns	ns
N*P*C	ns	ns	ns

Means in a column followed by same letter(s) within a treatment group are not significantly different, *, ** = Significant at 5% and 1% levels of probability, respectively

Data collected on fresh and cured bulb yield from the net plot was subjected to analysis of variance as described by Snedecor and Cochran [12] using a Microcomputer Statistical Programme (MSTAT) [13]. Significant differences were further analysed using Least Significant Difference Test (L.S.D.).

RESULTS AND DISCUSSION

The mean monthly minimum and maximum temperatures recorded across the two seasons ranged from 10.02 to 27.81°C and 25.40 to 40.53°C, respectively, while the mean relative humidity ranged from 34.78 to 52.83 percent. Laboratory analysis of soil samples from experimental site indicated the soil to be sandy loam, low in nitrogen, phosphorus and cation exchange capacity, moderate in available cations and acidic in reaction.

Fresh bulb yield: Results on spacing, planting date and cultivar effects on fresh bulb yield in 1997 and 1998 seasons and combined are presented in Table 1. Results indicated that plant spacing had significant ($p<0.05$) effect on fresh bulb yield throughout the study. It was found that increasing plant spacing from 5 to 10 cm increased fresh bulb yield, further increase to 20 cm resulted in significant decline in the yield. The highest fresh yield of 10534 kg haG^{1} was recorded at 10 cm spacing in 1998.

With regards to the effects of planting date on fresh bulb yield, it was found that the earliest planting date (Nov. 29) consistently resulted in significantly ($p<0.01$) higher fresh bulb yield than the later dates throughout the study. The highest fresh yield of 12810 kg haG^{1} was recorded at the earliest planting date in 1997. This signified the advantage of early planting in favour of larger fresh bulb yield.

There was significant ($p<0.05$) difference in the fresh bulb yield of the two cultivars in 1998 and combined, with ex-Lugu out yielding ex-Kofa. No significant interactions were observed.

Cured bulb yield: Data on the effects of plant spacing, planting date and cultivar on cured bulb yield in 1997 and 1998 dry seasons and combined are presented in Table 2. Plant spacing had significant effect on cured bulb yield in both seasons and combined. It was observed that as plant spacing was reduced from 20 to 10 cm there was significant increase in cured bulb yield; further reduction to 5 cm reduced the yield. The highest yield of 8430 kg haG^{1} was recorded from 10 cm spacing in 1998.

This indicated that close spacing of 10 cm had optimum effect on cured bulb yield. As spacing was increased, individual plant yield got better due to the declining level of interplant competition. But wider spacing meant less number of harvestable bulbs per unit area. Similar observations were reported by Duimovic and Bravo [14], Singh *et al*. [15], Lammerink [16], Aleksiev [8] and Babaji [9].

From the foregoing it is pertinent to observe that significant response was recorded on the effect of spacing on bulb yield. Widening the space between plant stands reduced interplant competition in favor of growth and yield factors, but reduced crop yield performance due to reduction in plant stand per unit area. This finding is in conformity with the work reported by Om and Srivastava [17], Noural [18], Lucero *et al*. [7], Rahim and Talukdar [19] and Lammerink [16]. Garlic crops planted at the widest spacing were found to give the maximum yield of bulbs per stand [19], but lower yield per unit area. This showed that an improvement in individual plants at wider spacing did not compensate for the reduction in yield due to a decrease in plant population. On the other hand, the closer spacing produced the highest yield but the quality (bulb size and weight) of produce may be adversely affected and may not attract good price in the market [15]. Kusumo and Widjajanto [6] in Indonesia obtained their best yields at spacing of 15×15 or 15×10 cm. In the Ukraine, Bogantirenko [20] obtained the highest yield at a spacing of 45×4 cm.

Planting date had highly significant effect on cured bulb yield throughout the study. A close observation of the data indicated significant decline in cured bulb yield with every two weeks delay in date of planting. The yields recorded in 1997 were 10675, 3070 and 2717 kg haG^{1} for the Nov. 29, Dec. 13 and 27 planting dates, respectively. This indicated the positive effect of early planting in favour of higher cured bulb yield. In a similar

Table 2: Cured bulb yield (kg haG^{1}) as affected by plant spacing, planting date and cultivar during 1997 and 1998 dry seasons and combined analysis at Sokoto

	Cured bulb yield		
Treatments	1997	1998	Combined
Nitrogen (kg haG^{1})			
0	6102	3584b	4843b
60	5911	6439a	5913a
120	6422	6692a	6557a
180	5956	5326a	5641ab
240	5658	5817a	5688ab
Significance	ns	**	*
L.S.D.	1252	1729	1056
Phosphorus (kg haG^{1})			
0	6028	5176	5602
22	5673	5901	5787
44	6328	5638	5983
Significance	ns	ns	ns
L.S.D.	970	1339	818
Cultivar			
Ex-Lugu	6324	5584	5954
Ex-Kofa	5695	5560	5627
Significance	ns	ns	ns
L.S.D.	792	1094	668
Interaction			
N*P	ns	ns	ns
N*C	ns	ns	ns
P*C	ns	ns	ns
N*P*C	ns	ns	ns

Means in a column followed by same letter(s) within a treatment group are not significantly different, *, ** = Significant at 5 and 1% levels of probability, respectively

report from Egypt, Maksoud *et al*. [21] and Shahien [22], observed that when cloves were planted on September 15th, October 1st or October 15th, planting on Sept. 15th produced higher yields than the planting on 1st and 15th October. In contrast to this however Nassar *et al*. [23] and Fouda *et al.* [24] working also in Egypt indicated that planting garlic on October 1st gave higher yield as compared with 1st September. Orlowski and Rekwska [25] while investigating the effect of planting date on two local garlic types in Sczecin (Poland) also observed that, early planting (20th Sept and 5th Oct) gave the highest marketable yield of 14 and 12 t ha^{-1}, respectively. Lipinski [26] also in Poland indicated that all the measured growth and yield indices studied were at their highest values with 25th Oct. planting. In Sudan Noural [18] found that garlic yields varied substantially from 1619 to 11123 kg ha^{-1} over a period of four seasons with three planting methods. He attributed the difference to temperature variations over time, higher planting rate resulted in higher yield but lower number of cloves per bulb. Also in Cordoba, Argentina Rendon *et al.* [27] obtained the highest yield (bulb with an average dry weight of 28.91 g) with white garlic planted early, Maksoud *et al.* [21] observed that under Egypian conditions suitable planting dates varies with location as follows Shakalan 1st to 15th September, Zagazig Sept. 15th to October 15th and Sids September 15th to 1st October. Under the conditions prevalent in different countries, the most suitable planting date for garlic was April 5th in Chile [28], Oct. 31st in Bangladesh [19], April 27th in New Zealand [29] and October 15th in India [15].

The effect of cultivar on yield performance was significant in 1998 and combined analysis. At these levels, the ex-Lugu significantly out yielded the ex-Kofa. The yields were 8024 and 5835 kg ha^{-1} for the two cultivars in 1998, respectively. Rendon *et al*. [27] in Cordoba, (Argentina) indicated that white garlic out yielded the red type. In Egypt Nassar *et al*. [23], Zaki [30] observed that the Chinese cultivar significantly out yielded the American and Egyptian cultivars. However Maksoud *et al*. [21] proved that the location where these cultivars were grown in Egypt, significantly affected their yield. They observed that Chinese cv. produced maximum yield at Shalkan and Zanazig, while the Egyptian cv. produced higher yield at Sids.

CONCLUSIONS

Based on the results obtained from this study it is suggested that garlic should be planted early in the dry season Nov. 29th to Dec. 13th and use inter plant spacing of 10 cm with ex-Lugu cultivar for maximum yield of garlic at Sokoto under similar soil and weather conditions as used in the trial.

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Productivity of Two Garlic (*Allium sativum* L.) Cultivars as Affected by Different Levels of Nitrogenous and Phosphorous Fertilizers in Sokoto, Nigeria

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Abstract: Two field experiments were carried out during the 1997 and 1998 dry seasons at the Usmanu Danfodiyo University,Teaching and Research *Fadama* Farm Sokoto [13° 01'N Latitude and 5° 15'E Longitude, 350 meters above sea level}, in order to study the response of two garlic (*Allium sativum* L.) cultivars to varying nitrogen and phosphorus levels. A factorial combination of five nitrogen levels (0, 60, 120, 180 and 240 kg N ha^{-1}), three phosphorus levels (0, 22 and 44 kg P ha^{-1}) and two garlic cultivars (ex-Lugu and ex-Kofa) were laid out in a Completely Randomized Block Design and replicated three times. Data was collected on fresh and cured bulb yield and analysed. Application of 120 kg N ha^{-1} produced the highest garlic yield. Increase in phosphorus levels from 0 to 44 kg P ha^{-1} had no significant effect on yield. The cultivars ex-Lugu and ex-Kofa were similar in yield performance.

Key words: Nitrogen % phosphorus % cultivar and garlic yield

INTRODUCTION

Garlic (*Allium sativum* L.) belongs to the family Alliaceae. Other crops in the family are onion (*A. cepa* L.), leek (*A. ameloprisum* L.), shallot (*A. asacloncum* L.) and chive (*A. schoenoprasum* L.). Garlic is the second most widely used of the cultivated bulb crops after onions. It is an erect annual herb that can reach a height of 75-90 cm and grows during dry and mild winter season [1].

Garlic is believed to have originated in Central Asia (India, Afghanistan, W. China, Russia etc) and spread to other parts of the world through trade and colonization [2, 3]. According to FAO [4], production of garlic stood at about 10 million tonnes per annum which is only about 10% that of bulb onions. China is the world largest producer followed by South Korea. The world average yield of garlic is about 10 t ha^{-1}, but can go up to 19 t ha^{-1}.

Garlic is rich in sugar, protein, fat, calcium, potassium, phosphorus, sulfur, iodine, fibre and silicon, in addition to vitamins. Its pungent flavor makes it used mainly as a spice, seasoning and flavoring for foodstuff involving both green tops and bulbs. Its medicinal value is also well recognized in the control and treatment of hypertension, worms, germs, bacterial and fungal diseases, diabetes, cancer, ulcer, rheumatism etc. Dehydrated garlic and extracts are fast replacing fresh bulbs for industrial and home usage in the production of drugs, insecticides and explosives [2].

Sokoto is among the leading garlic producing states in Nigeria. Production of the crop dates back several decades in the state. Garlic cultivation in Sokoto covers an area of over 11,277 ha producing about 73,908 metric tonnes per annum [5]. The main producing Local Government areas are Goronyo, Wurno, Gada, Gwadabawa, Rabah and Kware, where the crop is grown under irrigation during the cool dry season (Hamattan) in Nov-March. The traditional husbandry practices have resulted in 3-4 t ha^{-1} yield as against the world average of 10-15 t ha^{-1}. At off-season same quantity of garlic is usually sold at twice or three times the value of onions. It is mostly exported to other parts of Africa, Middle East, Asia and Europe through various trade routes. A number of studies in various parts of the world have shown that garlic production can be improved through appropriate cultural practices [6-8]. Unfortunately, there is dearth of information on garlic production in Nigeria, except for some recent work at Samaru on spacing, fertilizer and irrigation requirements [9-11].

It is in view of this background that this study was undertaken with the aim of exploring opportunities to

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improve the productivity of the crop through the choice of appropriate levels of nitrogenous and phosphorous fertilizers and suitable cultivar that maximizes the yield factors.

MATERIALS AND METHODS

Two field experiments were carried out during the 1997 and 1998 dry seasons (November to March) at Usmanu Danfodiyo University Teaching and Research *Fadama* Farm Sokoto, [13° 01'N Lat. 5° 15'E Long., 350 meters above sea level], in order to study the response of two garlic (*Allium sativum* L.) cultivars to varying levels of nitrogenous and phosphorous fertilizers.

The treatments consisted of five nitrogen levels (0, 60, 120,180 and 240 kg N ha^{-1}) and three Phosphorus levels (0, 22 and 44 kg P ha^{-1}) with two garlic cultivars (ex-Lugu and ex-Kofa). The treatments were factorially laid out in Randomized Complete Block Design replicated three times. Land preparation was carried out 14-18th November and planting 13th December of each year. Each gross plot was 1.5 m wide and 2.0 m long (3 m^2) consisting of ten rows, while the net plot was 0.9×1.4 m (1.26 m^2) consisting of six inner row. The cultivars used were ex-Lugu and ex-Kofa garlic cultivars. While the ex-Lugu is popularly grown in Lugu Village of Wurno Local Government area of Sokoto State whose mature cloves have light pinkish outer skin, the ex-Kofa is a local garlic cultivar popularly grown in Kofa Village in Bebeji Local Government area of Kano State whose mature cloves have light pinkish covering scale.

Prior to planting, garlic bulbs were split into the individual cloves that were soaked in water over-night. The cloves were planted upright with epical tip exposed at 10 cm inter row spacing according to the treatment structure. The field was irrigated before sowing in order to provide good clove-soil-water contact. Subsequent irrigations were given at 7 days intervals. Fertilizer was applied according to treatment structure in form of Urea (45-46%N), N.P.K. (20-10-10) and single super phosphate (18% P_2O_5 ha^{-1}). A basal dose of half the nitrogen rate and full doses of the phosphorus and potassium were applied at planting. The second half dose of the nitrogen was applied at four weeks after sowing.

There was no incidence of either insect pests or disease in the crop throughout the growth period in both seasons except for the nut sedge (*Cyprus rotundus*). Weeding was done thrice each season manually by hoe weeding and hand picking. Bulbs were harvested when the leaves turned yellowish green and had started withering. The harvested bulbs were spread in single layers in an open space in two weeks time for curing.

Data collected on fresh and cured bulb yield from the net plot was subjected to analysis of variance as described by Snedecor and Cochran [12] using a Microcomputer Statistical Programme (MSTAT) [13]. Significant differences were further analysed using Least Significant Difference test (L.S.D.).

RESULTS AND DISCUSSION

The mean monthly minimum and maximum temperatures recorded across the two seasons ranged from 10.02 to 27.81°C and 25.40 to 40.53°C, respectively, while the mean relative humidity ranged from 34.78 to 52.83 percent. Laboratory analysis of soil samples from experimental site indicated the soil to be sandy loam, low in nitrogen, phosphorus and cation exchange capacity, moderate in available cations and acidic in reaction.

Fresh bulb yield: Fresh bulb yield of garlic during 1997 and 1998 and combined analysis as affected by the treatments are shown in Table 1. Application of increasing rates of nitrogen had significant effect on fresh bulb yields. It was observed that in 1998 and combined increasing levels of nitrogen from 0 to 120 kg ha^{-1} resulted in significant increase in fresh bulb yield. Further increase to 240 kg N ha^{-1} reduced the yield. The highest fresh bulb yield of 8.4 ton ha^{-1} was recorded with 120 kg N ha^{-1} in 1998.

Application of varying rates of phosphorus had no significant impact on fresh bulb yield during the two seasons and combined analysis. The two cultivars were at par in fresh bulb yield during the two seasons and combined. No significant interactions were observed.

Cured bulb yield: Effects of nitrogen, phosphorus and cultivar on cured bulb yield of garlic in 1997 and 1998 seasons and combined analysis are shown in Table 2. Increasing levels of nitrogen had significant effect in cured bulb yield. In 1998 and combined analysis the cured bulb yield significantly increased with increase in nitrogen from 0 to 60 and 120 kg N ha^{-1}. Higher dosage of 180 and 240 kg N ha^{-1} reduced the bulb yield. The highest yield of 6.7 ton ha^{-1} was recorded with 120 kg N ha^{-1} in 1998. This is in contrast with the report of Sadaria *at al*. [14] working on garlic cv G1 in Gujarat India, who reported that different nitrogen treatments had no significant effects on bulb yield. Similarly Kibreab and Hiranburana [15] in a two-year trial, carried out in

Table 1: Fresh bulb yield (kg haG¹) as affected by rate of nitrogen, phosphorus and cultivar during 1997 and 1998 seasons and combined analysis at Sokoto

	Fresh bulb yield		
Treatments	1997	1998	Combined
Nitrogen (kg haG¹)			
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60	7522	8047a	7785a
120	8140	8364a	8243a
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Significance	ns	ns	ns
L.S.D.	1214	1675	1023
Cultivar			
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Significance	ns	ns	ns
L.S.D.	991	1367	835
Interaction			
N*P	ns	ns	ns
N*C	ns	ns	ns
P*C	ns	ns	ns
N*P*C	ns	ns	ns

Means in a column followed by same letter (s) within a treatment group are not significantly different, *, ** = Significant at 5% and 1% levels of probability, respectively

Table 2: Cured bulb yield (kg haG¹) as affected by rate of nitrogen, phosphorus and cultivar during 1997 and 1998 seasons and combined analysis at Sokoto

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Thailand compared 4 levels of N-fertilization and reported that in both years, there was no response to increasing levels of nitrogen.

Aliudin [16] in Indonesia, using varying rates of nitrogen reported that the relationship between nitrogen rate and bulb yield (dry weight) was quadratic with the maximum yield at 172.81 kg N haG¹. In Chile Escaff and Aljarou [17] reported that 90 kg N haG¹ had no effect on yield or quality of garlic. But in a second fertilizer experiment with varying rate of nitrogen, the application of up to 150 kg haG¹ increased the yield and quality of the bulbs. Maksoud *et al.* [18] from Egypt also reported significant favourable effects of nitrogen application on yield. These workers observed that the addition of N at 360 kg haG¹ increased the yield of cured marketable bulbs from 12.4 to 20.5 t haG¹ in one trial and from 13.6 to 17.3 t haG¹ in another trial.

In Chile, Ruiz [19], reported that increasing rates of applied nitrogen from 0 to 150 kg haG¹ increased bulb yield from 4.6 to 10.6 t haG¹. The bulb quality also increased with increasing nitrogen application up to 225 kg haG¹. Carvalho *et al.* [20] in Brazil in a trial with 3 rates of applied N (0-120 kg haG¹), 4 rates of K (0-160 kg haG¹), observed various effects of N and K reported that total and marketable bulb yields were affected only by N. The highest yields were obtained with 70-76 kg N haG¹. Arboleya and Garcia [21] from Canelones, Uruguay in a trial with garlic with N at 0, 75, 150 and 225 kg haG¹ observed that marketable bulb yield increased from 4.66 t haG¹ at 0 kg N haG¹ to 8.04 t haG¹ at 225 kg N haG¹. At Samaru (Nigeria), Babaji [9] observed that N application increased almost all growth and yield parameters of garlic significantly and that the maximum yield of 15 t haG¹ was recorded with 90 kg N haG¹. In

another trial [22], the growth and yield parameters increased with increasing N rates up to 150 kg N haG¹; higher rates of N to 225 kg haG¹ decreased them. Bichi [10] on the other hand, recorded significant increase in bulb yield with the application of 75 kg N haG¹ while clove weight increased only at 150 kg N haG¹ beyond which there was a significant reduction.

Application of increasing levels of phosphorus had no significant effect on cured bulb yield during the study. This is in agreement with the findings of Escaff and Aljaro [17], they recorded no significant effect on the growth and yield of garlic with the application of 41.11 kg P haG¹ during bulbing and root growth. Minard [23] on the other hand, reported 114.75 kg P haG¹ as optimum for increased final bulb yield among other parameters. He attributed this effect to the influence of phosphorus on root development, which led to effective nutrients uptake and water absorption. Borabash and Kochina [24] also recorded significant increase in assimilating leaf area; photosynthetic productivity and final bulb yield with the application of 39.27 kg P haG¹ to garlic crop. Hilman and Noordiyati [25], however, reported that several levels of phosphorus tested on garlic did not significantly affect most growth and yield parameters. However a significant increase in bulb dry weight was recorded with the application of 150 kg haG¹ of triple super phosphate. Minard [23] recorded the highest garlic bulb yield with the application of 210 kg N haG¹ and 114.75 kg P haG¹. He reported that the increase was as a result of favourable effects of both nitrogen and phosphorus on growth and development of the crop. Escaff and Aljaro [17] recorded significantly higher bulb yield in garlic with the application of 150 kg N haG¹ in combination with 32.72 kg P haG¹. Hilman and Noordiyati [25] also recorded significantly higher yields of underground bulbs of garlic among other parameters with the application of 90 kg of N and 39.27 kg P haG¹. The cultivars ex-Lugu and ex-Kofa cured bulb yields werenot significantly different in both seasons and at combined level. No significant interaction was observed.

CONCLUSIONS

Based on the results obtained from this study it is suggested that a combined application of 120 kg N haG¹ and 22 kg P haG¹ for both the ex-Lugu and ex-Kofa cultivars can result in good yield of garlic in Sokoto under similar soil and weather conditions as used in the trial.

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Genotype by Environment Interaction for Cowpea Seed Yield and Disease Reactions in the Forest and Derived Savanna Agro-Ecologies of South-West Nigeria

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Abstract: Eight cowpea varieties were evaluated for two years in two locations within the south-western Nigeria. The locations are Ibadan (forest) and Ilora (derived guinea savanna). The cowpea varieties were evaluated for their field reactions to cercospora leaf spot and brown blotch fungal diseases. The effect of genotype x environment (g x e) interaction on seed yield was also investigated with the aim of identifying location specific genotypes for each of the two environments. Significant location, genotype and g x e interaction effects were observed for seed yield, days to 50% flowering and the cowpea reactions to the two diseases. Significantly higher seed yield was obtained at Ilora with average yield advantage of 64.87% over Ibadan. Average grain yield at Ilora was 1.22 t ha^{-1} while that of Ibadan was 0.74 t ha^{-1}. Incidence and severity of the two diseases were significantly higher at Ibadan than Ilora. Using AMMI analysis, suitable cowpea varieties were identified for each of the two environments. It was observed that cowpea varieties found to be adapted to Ibadan (forest) environment had low yield potential, this calls for the development of high yielding and disease resistant cowpea genotypes suitable for the forest environment.

Key words: Cowpea % genotype % environment % fungal diseases % Nigeria

INTRODUCTION

Genotype by environment interaction is an important consideration in crop improvement since relative performance of genotypes often changes from one environment to another. Genotype x environment interaction reduces association between phenotypic and genotypic values and thus, plant that performs well in one environment may not necessarily perform well in another environment [1]. Year, location and the climatic factors of a place such as rainfall, temperature and soil properties often affect crop production by interacting with disease causing organisms.

Cowpea is an important component of the food intake of the less developed countries of the world because of its high protein content [2]. The crop is susceptible to a range of pathogens whose importance varies with ecological zone. Brown blotch and cercospora leaf spot diseases are the most important fungal diseases limiting cowpea production in south-west Nigeria [3]. Brown blotch is caused by *Colletotrichum capsici* (Syd) Butler and Bisby. The disease is characterized by purple brown discoloration on the pods. Discoloration may also be observed on petioles, leaf veins and peduncles. Pod infections leads to distortion and maldevelopment of the pods [4, 5]. Cercospora leaf spot disease is caused by two fungi namely *Cercospora canescens* Ellis and martia and *Mycospharella cruenta* Lanthanus. It is characterized by irregular reddish lesions scattered all over the foliage. It can lead to severe premature defoliation [6].

Many of the improved cowpea varieties currently cultivated in the country are highly susceptible to these two diseases [3]. For high productivity, a crop variety being considered for recommendation in a particular environment should be resistant or tolerant to the prevailing diseases in that environment. The g x e interaction studies is of paramount importance in the specific environments in which the genotypes are to be grown [7]. Hence the objectives of this study were: (i) to evaluate the field reactions of the eight cowpea genotypes to cercospora leaf spot and brown blotch fungal diseases (ii) to investigate the effect of g x e interaction on seed yield in order to identify suitable

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high yielding cow pea varieties for each of the two environments.

MATERIALS AND METHODS

Eight cowpea varieties were planted in two locations within the south-western Nigeria for two years (2003 and 2004). The two locations are Ibadan and Ilora. Ibadan lies within the humid rainforest zone with latitude/longitude 7° 22' N/3 55'E, while Ilora falls within derived savanna agro-ecology with latitude/longitude 7° 45 'N/3° 55 'C. The cowpea varieties evaluated are IT90K-277-2, IT96D-610, IT84S-2246 and TVx 3236 [all from the International Institute of Tropical Agriculture (IITA)]; others are Ife brown, Ife-BPC and Ife-98-12 [from the Institute of Agricultural Research and Training (IAR and T)] and Erushu which is a local variety.

At each location, each of the eight cowpea varieties was planted in a five-row plot of 3 x 3m, replicated three times at a spacing of 60 x 30 cm. The trials were protected from insect attack by the application of cypermethrin + dimethoate (Sherpa plus) insecticide which was applied at the rate of 50g a.i. /ha three weeks after seedling emergence and twice after anthesis to control insect attack. No fungicide was applied and the fields were kept clean through manual weeding.

Between 6 and 7 weeks after planting the cowpea varieties were observed for the natural development of symptoms of cercospora leaf spot and brown blotch diseases. Incidence of each of the diseases on each cowpea variety was assessed by counting the number of plants showing symptoms of each of the two diseases and expressed as percentages. Disease severity scores used for the two diseases were based on a scale of 1-5. For cercospora leaf spot, 1 = no symptom, 2 = scattered leaf spots not more than 3 spots per leaf on few leaves, 4 = many spots on few leaves, 5 = many spots on most leaves with yellowing and defoliation occurring. For brown blotch disease, 1 = no symptoms, 2 = mild symptoms confined either to the stem or to the base and tip of the peduncle, 3 = stems, leaf veins and pods with moderate blotching but without distortion, 4 = heavy blotching of pods with some distortion, 5 = severe pod damage. Number of days to 50% flowering was also recorded for each variety. At harvest, dry pods from the middle three rows of each plot were harvested together, threshed and seed yield per plot was determined from which seed yield per hectare was estimated.

Data collected were subjected to analysis of variance, Duncan Multiple Range Test and simple linear correlation. In addition, yield data from the two environments (location by year combined) of the eight cowpea varieties were subjected to Additive main effects and multiplicative interaction model (AMMI) analysis using MATMODEL version 2.0 computer package [8] and biplot drawn for the identification of specific genotypes for each of the two environments.

RESULTS AND DISCUSSION

The results of the analysis of variance are shown in Table 1. Number of days to 50% flowering, incidence and severity of cercospora leaf spot disease varied between the two years. Significant location and variety effects were observed for all the parameters. Year x genotype interaction effect was significant for number of days to 50% flowering and the incidence of brown blotch disease indicating that the degree of susceptibility of the cowpea varieties to brown blotch disease differed between the two years. Location x genotype interaction effect was significant for all the parameters except for the number of days to 50% flowering. Year x location x genotype effect was also significant for number of days to 50% flowering, seed yield and incidence and severity of cercospora leaf spot disease. Differential behaviour of cowpea genotypes to varying environments have been reported previously [9, 10].

Average performance of the cowpea varieties and their reactions to the two diseases across the two locations are shown in Table 2. Number of days to 50% flowering ranged between 37.72 to 44.92 days with the local variety (Erushu) being the earliest to flower. The variety IT84S-2246 had the highest seed yield of 1.18 t haG^{1} with Ife-98-12 having the least seed yield of 0.83 t haG^{1}. The most susceptible varieties to cercospora leaf spot were Ife BPC and IT84S-2246. The most resistant variety to the disease appeared to be IT90K-277-2 with disease incidence of 5.75% and severity score of 1.67. The cowpea varieties Ife BPC and Ife brown were the most susceptible to brown blotch disease with the percentage incidence of 19.0% and 18.75% respectively.

Number of days to 50% flowering and seed yield in each of the two locations are shown in Table 3. Number of days to 50% flowering ranged between 37.67-44.83 days at Ibadan and from 38.17-45.30 days at Ilora. Seed yields of the cowpea varieties were significantly higher at Ilora than at Ibadan and it ranged between 0.98-1.52 t haG^{1} at Ilora and 0.61 to 0.93 at Ibadan. The best yielding variety at Ilora was IT84S-2246 while the highest yielding variety

Table 1: Mean square values for cowpea seed yield and disease reactions in two locations

Source of variation	Days 50% flowering	Yield	Cecospora incidence	Cercospora severity	Brown blotch incidence	Brown blotch severity
Year	26.04**	0.00003	2271.76**	10.01**	42.67	0.38
Location	12.04	5.49**	490.51**	7.59**	1107.04**	20.17**
Genotype	63.00**	0.15**	349.7**	2.02**	396.31**	2.45**
Year x Loc	54.96**	0.12	82.51	0.26	9.38	0.04
Year x Gen	15.85**	0.11	36.74	0.44	107.33**	0.35
Loc x Gen	1.23	0.17**	219.20**	1.92**	168.52**	1.05**
Y x L x G	14.76*	0.14**	99.58**	0.55**	13.94	0.16

*, **, Significant at p<0.05 and 0.01 respectively

Table 2: Average performance and disease reactions of the 8 cowpea varieties

Variety	Days to 50% flowering	Yield (t haG¹)	Cercospora incidence (%)	Cercospora severity	Brown blotch incidence (%)	Brown blotch severity
1 Erushu	37.92e	0.91bc	6.5d	1.58c	9.92c	2.00c
2 IT90K-277-2	44.92a	0.99ab	5.75d	1.67bc	1.58d	1.25d
3 Ife brown	42.75c	0.92bc	16.75ab	2.42a	18.75a	2.75a
4 IT96D-610	43.00bc	1.10ab	17.83ab	2.5a	15.83ab	2.08c
5 IT84S-2246	44.17ab	1.18a	19.00a	2.42a	17.08ab	2.25bc
6 Ife-98-12	41.17d	0.83cd	12.00cd	1.75bc	13.83ab	2.08a
7 TVx 3236	44.67a	0.95bc	13.58bc	2.00b	13.00bc	2.33bc
8 Ife BPC	43.42bc	0.95bc	19.33a	2.58a	19.00a	2.58ab
Mean	42.75	0.98	13.84	2.11	13.63	2.17
SEM	0.24	0.03	0.86	0.06	0.63	0.05

Numbers in the same column followed by the same letter(s) are not significantly different at p<0.05

Table 3: Number of days to 50% flowering and cowpea seed yield in each of the two locations Ilora and Ibadan

	Days to 50% flowering		Yield (t haG¹)	
Variety	1	2	1	2
1 Erushu	38.17c	37.67d	1.15ab	0.67ns
2 IT90K-277-2	45.00a	44.83a	1.38ab	0.61
3 Ife brown	43.00ab	42.50bc	1.06bc	0.78
4 IT96D-610	43.33ab	42.67abc	1.44a	0.76
5 IT84S-2246	44.83a	43.50abc	1.52a	0.83
6 Ife-98-12	41.00bc	41.33cd	1.05bc	0.61
7 TVx 3236	45.33a	44.00ab	1.17ab	0.74
8 Ife BPC	44.17ab	42.67abc	0.98c	0.93
Mean	43.10	42.40	1.22	0.74
S.E.M	0.39	0.25	0.04	0.03

Numbers in the same column followed by the same letter(s) are not significantly different at p<0.05. 1 = Ilora, 2 = Ibadan, ns = not significantly different at p<0.05

at Ibadan was Ife-BPC. The incidence and severity of the two diseases were however higher at Ibadan (Table 4). Average incidence of cercospora leaf spot at Ilora and Ibadan were 11.58 and 16.0% respectively while those of brown blotch disease were 10.23 and 17.02% respectively. The most susceptible varieties to cercospora leaf spot disease at Ilora were Tvx 3236 and Ife-BPC, while IT84S-2246, Ife-BPC and Ife brown varieties were the most susceptible at Ibadan (Table 4). The most susceptible variety to brown blotch disease at Ilora was Ife-BPC while Ife brown, IT96D-610 and IT84S-2246 were the most susceptible varieties to the disease at Ibadan. Ife brown had the highest disease severity score of 3.67 at this location (Table 4). In this study, environment 1 (Ilora) appeared to be higher yielding compared with Ibadan having seed yield advantage of 64.87% with less incidence and severity of the two diseases which favoured cowpea production (Tables 3 & 4).

Although IT84S-2246 was found to be susceptible to the two diseases at the two locations, it was still highly productive; the seed yield potential of this variety reduced the effects of the diseases on the yield. The variety, IT90K-277-2 appeared to possess field resistance to both cercospora leaf spot and brown blotch diseases at the two locations. The actual level of resistance has to be ascertained with artificial inoculation. The local variety Erushu also combined early maturity with high level of field resistance to the two diseases. It would be incorporated into further breeding programme to improve

Table 4: Reactions of the cowpea varieties to cercospora leaf spot and brown blotch diseases in each of the two locations Ilora and Ibadan

	Cercospora incidence (%)		Cercospora severity		Brown blotch incidence (%)		Brown blotch severity	
Variety	1	2	1	2	1	2	1	2
1 Erushu	7.33^{ns}	5.67c	1.66^{ns}	1.50b	9.17bc	10.67c	1.83ab	2.16c
2 IT90K-277-2	7.17	4.33c	1.67	1.67b	1.33c	1.83d	1.17b	1.33d
3 Ife brown	11.00	22.50ab	1.83	3.00a	11.67ab	25.83a	1.83ab	3.67a
4 IT96D-610	13.33	22.33ab	2.17	2.83a	6.33bc	25.33a	1.50ab	2.67bc
5 IT84S-2246	9.83	28.17a	1.67	3.17a	10.50b	23.67a	1.67ab	2.83bc
6 Ife-98-12	10.50	13.50bc	13.50	1.50b	2.00ab	14.67bc	1.83ab	2.33c
7 TVx 3236	17.50	9.66c	2.33	1.67b	10.67b	15.33bc	2.00ab	2.67bc
8 Ife BPC	16.00	22.67ab	1.83	3.33a	19.17a	18.83ab	1.83ab	3.33ab
Mean	11.58	16.10	1.83	2.40	10.23	17.02	1.71ab	2.62
SEM	1.32	1.10	0.09	0.09	0.97	0.81	0.06	0.08

Numbers in the same column followed by the same letter(s) are not significantly different at p<0.05, 1 = Ilora, 2 = Ibadan, ^{ns} = not significantly different at p<0.05

Table 5: Correlation coefficients of the incidence and severity of the diseases with seed yield

S/N		1	2	3	4	5	
1	Days to 50% flowering	x					
2	Yield	0.16	x				
3	Cercospora incidence	0.02	-0.0	-0.01	x		
4	Cercospora severity	-0.01	-0.10	-0.09	84.0**	x	
5	Brown blotch incidence	-0.07	-0.20*	0.38**	0.43**	x	
6	Brown blotch severity	-0.05	-0.31**	0.40**	0.53**	0.78**	x

$^{*, **}$, Significantl at p<0.05 and 0.01 respectively

its yield potential. Traditional varieties are known to be good sources of desirable characters such as disease resistance.

The results of the correlation analysis are shown in Table 5. All the disease parameters are highly positively correlated (p<0.01) with one another. This significant positive correlation between brown blotch and cercospora leaf spot diseases indicate that selection for resistance to either of the disease could result in progress in resistance to the other. Although, the incidence and severity of cercospora leaf spot disease were negatively correlated with seed yield, the correlation was not significant. However, there were significant negative correlations between seed yield and incidence and severity of brown blotch disease indicating that high susceptibility of cowpea to this disease would significantly reduce seed yield. This calls for the cultivation of brown blotch disease resistant varieties in the endemic areas to alleviate yield loss due to the disease. Yield loss incurred from brown blotch disease in northern guinea savannah of Nigeria was estimated to range from 46-75% [11, 12]. The identified resistant variety IT90K-277-2 could be recommended to farmers in the endemic areas. The negative correlation between cowpea seed yield and incidence and severity of the two diseases evaluated was partly responsible for the low seed yield of the cowpea varieties obtained at Ibadan as these diseases were more prevalent in that environment. Other factors that could be responsible for low yield in the forest environment may include rainfall distribution temperature and soil properties.

Table 6: AMMI analysis of variance for cowpea seed yield evaluated in two environments

Source of variation	Degree of freedom	Sums of square (SS)	Mean square	SS%
Total	47	4.55	0.97	
TRT	15	3.83	0.25**	
Gen	7	0.45	0.06*	9.89
Env	1	2.79	2.79**	61.32
G x E	7	0.60	0.09**	13.19
IPCA1	7	0.60	0.09**	
Error	32	0.73	0.02	

*, **, Significant at p<0.05 and 0.01 respectively

The results of the AMMI analysis are shown in Table 6. Only one Interaction Principal Component Axis (IPCA) was produced as there were only two environments involved in the analysis. The result showed

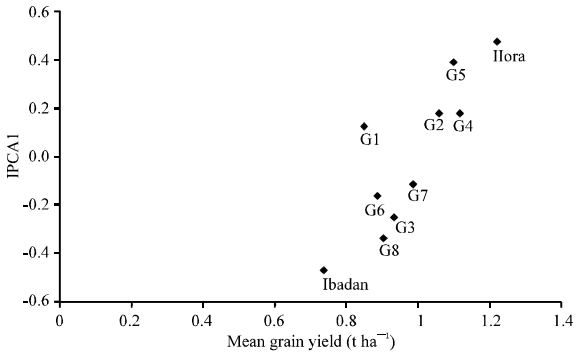

Fig. 1: Distribution of the 8 cowpea varieties between the two sties

that environment (obtained as location by year combined), genotype and genotype x environment interaction were significant for seed yield (Table 6). Environment alone accounted for 61.32% of the total sums of square indicating that differences in yield potential of the two environments was responsible for most of the observed variation in seed yield. While genotype and g x e interaction were responsible for 9.89% and 13.19% of the total sums of square respectively (Table 6). Similar observations have been reported in the past [13, 14]. Each of the two sites exhibited distinct effects on the performance of the cowpea varieties. From Fig. 1, Ilora (derived savannah) was high yielding with positive interaction while Ibadan (forest) appeared to be low yielding for cowpea production with negative interaction. High yielding cowpea varieties IT84S-2246 (G5), IT96D-610 (4), IT90K-277-2 [3] with mean seed yield above the grand mean were more suited to the Ilora environment, while cowpea varieties Ife BPC (G8) and Ife brown (G3) were more adapted to the Ibadan environment. The cowpea variety TVx 3236 (G7), though high yielding, had negative interaction while the local variety (G1) had positive interaction but low yielding. It was observed that the cowpea varieties identified to be adapted to Ibadan environment had low yield potentials. Continuous efforts have to be made to breed for high yielding genotypes with high level of resistance to the prevailing diseases in the forest environment. Although forest environment has low yield potential for cowpea production, farmers in the area still cultivate the crop either as catch crop after the harvest of the main crops such as maize, yam, cassava, etc. or planted in intercrop with the main crops.

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The Measurement of Nitrate and Nitrite Content in Leek and Spinach Sampled from Central Cities of Mazandaran State of Iran

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Abstract: Vegetables especially leafy ones are the major source of nitrate and nitrite in human diet. Because of the potential health hazards result in high intake of nitrate and nitrite, determination of these ions content in vegetables the concentration has been considered and measured in many countries. The aim of this research was to determine the concentration of nitrate and nitrite in leek and spinach sampled from three central cities of Mazandaran state (IRAN). So, 12 samples of each vegetable have been gathered from farmlands of 4 geographical regions in each city. At last, we assessed 36 leek samples and 36 spinach samples. The measurement was based on ISO Method (NO.6635). Data has been analyzed with T-test and ANOVA.According to the results, the average of nitrate and nitrite content in all of the samples was less than standard limits. Qaemshahr-sampled leek had significantly higher nitrate content than 2 other cities ($p<0.01$), on the other hand, spinach sampled from Sari had significantly lower nitrate content than 2 other cities ($p< 0.05$).

Key words: Mazandaran % Nitrite % Nitrate % Vegetables

INTRODUCTION

In recent years, an increasing interest in the determination of nitrate levels in food products has been observed, essentially due to the potential reduction of nitrate to nitrite, which is known to cause adverse effects on human and animal health. In fact, nitrite in may react with secondary amines to form toxic and carcinogenic nitrosamine compounds. In addition, nitrite is also known to cause methemoglobinemia (oxygen deficiency) in infants [1, 2, 3]. Fresh and processed vegetables, specially leafy ones, have often been cited as the major sources of dietary nitrate intake, owing to their nitrate accumulation capacity [4, 5, 6]. Nitrite content in vegetables is usually very low as compared to nitrate [7]. Then, in the past few years, efforts have been made to study nitrate accumulation in vegetables and the factors that might influence its occurrence. Actually, the amount of available nitrate in soil (which may be related to the amount of commercial fertilizers applied) appears to be a major factor determining the nitrate concentration level in vegetables [5, 8]. In 1997, to eliminate trade barriers across the European Union, European Commission Regulation (EC) No. 194/97 set harmonized maximum levels for nitrate in some vegetables. The limits vary according to season, with higher nitrate levels permitted in winter-grown vegetables [9, 10].

At the end, the objectives of this project were to 1) determine the amount and the variability of the nitrate and nitrite content in leek and spinach grown in farmlands of Sari, Qaemshahr and Babol cities by UV-Visible Spectrometry, 2) evaluate the relative safety of these leafy vegetables based on the maximum levels of European Commission Regulation (EC) No. 194/97.

MATERIALS AND METHODS

Sampling: Vegetables were sampled from local farmlands of Sari, Qaemshahr and Babol between Sep.23 to Nov.23, 2006. Four geographical areas were chosen in each of cities (north, south, east and west) and in each area, three samples of each vegetable were gathered randomly. Samples were carried to laboratory in iceboxes and were analyzed as soon as possible. If storage was necessary, samples were stored at -4°C.

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Apparatus:

- Spectrophotometer UV/Visible, model: Ultrospec 4000 for use at 538 nm, providing a light path of 1 cm or longer.
- Reduction column purchased from Kimble Kontes Co. (USA), filled by Cadmium coarse powder 0.3-0.8 mm (Merck). Preparation and efficiency test of cadmium column was based on ISO Method (No.6635) [11].

Reagent and solution: All chemicals used, were of analytical reagent grade purchased from Merck Co. (Germany). Deionized water (with a specific conductance of less than 0.1 µS cm^{-1}) was used throughout.

Procedure: The measurement method was based on ISO Method (NO.6635) [11]. 10 g from 200 homogenized vegetables weighed precisely, added hot deionized water (80°C) and 5 ml saturated tetra borate and heated on water bath for 15 min, After that the aqueous extract was clarified with Potassium hexacyanoferrate and Zinc acetate, slurry transferred to 200 ml volumetric flask and diluted by deionized water, then filtrated.

Determination of nitrite content: 10 ml aliquot of filtrate was transferred to a 50 ml flask. After developing of purple color due to addition of sulfanilamide and *N*-(1-naphthyl)ethylene diamine dihydro chloride,diluted to volume 50 ml and measured the absorption in 538 nm on the standard curve with linear equation: $y = 0.015 + 0.022x$, (r^2=0.999).

Determination of nitrate content: A second 10 ml aliquot of filtrate was mixed with buffer (pH=9.6) and passed through the reduction column. Column was washed with ca??15 ml deionized water. The eluate and wash were collected in a 50 ml volumetric flask and continued the rest as like as nitrite procedure.

Statistical Analysis: The nitrate and nitrite content in two vegetables of three cities analyzed descriptively and $p<0.05$ set as significant differences to one-way ANOVA and T-test of means. All statistical analyses were done by SPSS software for windows Ver.14.

RESULTS

The average of nitrate content in spinach and leek which sampled from four different geographical areas in each city was investigated and compared together (Table 1).

Table 1: Summary of nitrate content in spinach and leek (NO_3 mg kg^{-1} FW) in four geographical areas of three cities ($p<0.01$).

			Spinach	Leek
City	Region	N	Mean ± S.D.	Mean ± S.D.
Babol	North	3	401 ± 60	0.00
	West	3	351 ± 10	0.00
	South	3	310 ± 61	0.00
	East	3	188 ± 9	0.00
Qaemshahr	North	3	457 ± 44	86 ± 14
	West	3	364 ± 28	60 ± 3
	South	3	194 ± 23	83 ± 4
	East	3	439 ± 37	79 ± 22
Sari	North	3	24 ± 5	0.00
	West	3	150 ± 3	0.00
	South	3	346 ± 36	0.00
	East	3	282 ± 27	0.00

Table 2: The total average of nitrate content in spinach and leek (NO_3 mg kg^{-1} FW) of 3 cities ($p<0.05$)

		Spinach*	Leek**
City	N	Mean ± S.D.	Mean ± S.D.
Babol	12	313 ± 89	0.00
Qaemshahr	12	364 ± 112	77 ± 16
Sari	12	223 ± 129	0.00

* $p< 0.02$, ** $p <0.01$

According to the nitrate content in spinach, there was a significant difference between east of Babol, south of Qaemshahr, north of Sari and the other 3 regions of each city ($p<0.01$). But no significant difference was found in the average of nitrate content between leek samples of Qaemshahr.

Total average of nitrate content in spinach was 312, 363 and 223 (NO_3 mg kg^{-1} FW), in Babol, Qaemshahr and Sari, respectively ($p<0.01$). On the other hand, leek samples contained 0.0, 76, 0.0 mg/kg FW of nitrate, in Babol, Qaemshahr and Sari, respectively (Table 2). Qaemshahr-sampled leek had significantly higher nitrate content than 2 other cities ($p<0.01$) and spinach sampled from Sari had significantly lower nitrate content than 2 other cities ($p< 0.05$).

The amount of nitrite in all samples of both vegetables (spinach and leek) in all 3 cities was very low and not detectable.

DISCUSSION

The tissue nitrate levels for spinach and leek recorded in this study are comparable to the levels reported elsewhere [12-14]. A research was done in

Esfahan-IRAN (Fall 1998- Summer 1999) showed that the mean of nitrate in some leafy vegetables was 287.9 ppm [15]. The European Union set maximum limit for nitrate and nitrite in fresh spinach (harvesting during 1 November to 31 March) is 3000 and 0.0 ppm, respectively [9]. In addition, this limit for nitrate and nitrite in leek is 2500 and 0.0 ppm respectively. Corresponding to the results (Table 1 and 2) the average of nitrate and nitrite concentration in all spinach and leek samples of three cities was less than the maximum levels that specified by European Commission Regulation. This means that the investigated vegetables are safe for consumption. According to Table 1, the mean of nitrate content in spinach samples from north of Babol, north and east of Qaemshahr, east of Sari were more than other areas in each city. Also Qaemshahr-sampled spinach and leek had more nitrate content than other two cities. These results can be explained as follows:

- Vegetable nitrate levels are affected by the rate and type of nitrogen fertilizers applied and by soil nitrification activity, soil texture and harvest time [16].
- Water that use in this areas is from flowing water unlike the other areas that use well water for irrigation.
- uch rain and over flowing water of other areas to the mentioned areas, cause to accumulation of nitrate [17].

Suggestion:

- easurement of nitrate and nitrite in agricultural products should be regularly performed according to per capita consumption of fertilizer.
- ccording to have not any standard level for vegetables nitrate content in IRAN, seems necessary to establish specific maximum standard level considering to different condition in our country.
- ertilizing and irrigation practices that produce vegetables with low nitrate content compatible with optimum yield must be developed [18, 19].
- sing methods such as preplant soil nitrate testing, compost based fertility management, afternoon to evening harvest, may reduce nitrate concentration in vegetables [20, 21].
- ducation the people, about the better preparation and storage of edible part of vegetables (Like petiole removal).

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Jojoba Oil as a Novel Coating for Exported Valencia Orange Fruit Part 1: The Use of Trans (Isomerized) Jojoba Oil

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Abstract: The first utilization of Trans (Isomerized) Jojoba Oil for coating of Valencia orange fruits (*Citrus sinensis*), as a novel waxing material and an alternative to the usual wax used in citrus packinghouses for export fruits was studied. Trans Jojoba Oil (TJO) was prepared via isomerization of Jojoba Oil under optimized conditions to fulfill the coating properties. Hand coated fruits were stored at 5°C up to 60 days before transferred to 20°C for one week as shelf-life period. Waxed orange fruits were compared with untreated ones (control) and also those treated with Exported wax (E. wax) as a simulation of commercial wax. Fruit quality characteristics (weight loss, decay percentages, respiration rate, ssc, acidity and ascorbic acid content) were evaluated periodically at removal from cold storage and after holding at 20°C. TJO treatments were markedly reduced the weight loss and respiration rate than that of control fruits. Whereas, Equal significant values of E-wax treatment and the highest TJO concentration (30%) were observed. Moreover, coated fruit stored for two months at 5°C withstand free from microbial pathogenic incidence, but with softening symptoms ranged between 3.49-5.36% with inversely proportional to the isomerized Jojoba concentrations. Although, SSC showed insignificant differences due to TJO treatments throughout storage period, titratable acid content showed lower decrease percent as TJO concentrations increased, but with a slight increase than its initial content at harvest. Ascorbic acid (Vit. C) content had significant decrease by expanding cold storage period with slight loss in fruits coated with highly concentrations of isomerized jojoba wax. In conclusion, data indicated that 20-30% of Trans Jojoba Oil concentrations proved to be the most capable treatments in maintaining Valencia orange fruit quality up to 60 days storage at 5°C. Moreover, TJO was found efficient enough for coating fruits equal to the export wax and more promising than any other coating materials.

Key words: Valencia orange % jojoba oil % isomerized (Trans) jojoba oil % coating % cold storage % fruit quality % simulation handling

INTRODUCTION

In fruit handling process in the packinghouses, the natural waxes in skin fruit are removed. It is imperative that these natural protectants are replaced by different coatings. Citrus fruit are commonly waxed to give the fruit a shiny, attractive appearance and excellent barrier properties [1-3]. Fruit coatings are used commercially to improve outward appearance; fruit coating and protective film treatments also modify the internal atmosphere of fruit and, as much, have great potential as shelf life extending treatments for apples and other fruits [4-6].

Wax coatings have been shown to extend postharvest quality of fruit and vegetable crops by limiting gas exchange and reducing water loss, skin discoloration, fruit deterioration and should not cause partial anaerobic conditions [7-9]. Since, most waxes used in fruit coating are mainly waxes belong to the non-polar lipid classes and prevent the molecule spreading. It is objective to use other forms of waxes such as Jojoba Oil, which modified via isomerization to produce Trans Jojoba Wax [10-12].

As far as we know, waxing of Valencia orange fruit with Trans Jojoba Oil (TJO) has not been utilized for such

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citrus fruits. It is considered as a novel waxing material for the Valencia orange fruits in citrus packinghouses for export, as an alternative to the usual wax coatings, Trans Jojoba Oil (Isomerized) can be used in this respect [2, 12-14]. Converting the double bonds of Jojoba Oil, which is commonly known as liquid wax, from cis to trans diastereomers produces a soft wax with a low melting point. The results of the oxidation stability of the oil indicate excellent stability [16]. The Trans-Isomerized oil and other Jojoba oil derivatives have a wide range of industrial uses [6, 15-17].

The present study focuses on the evaluation of the influence of Trans Jojoba Oil (TJO) concentrations as conventional coating wax in maintaining Valencia orange fruit quality during cold storage and shelf-life period as a simulation of handling and shipment of citrus fruits for export.

MATERIALS AND METHODS

Fruit: Valencia orange fruit (*Citrus sinensis*) were obtained from a private orchard (Dina) in Cairo-Alex road district, Giza Governorate. Fruits were picked from 15 years old trees grown in sand-loam soil and were similar in growth and received common horticulture practices. Mature orange fruits, undamaged, free from apparent pathogen infection, uniform in shape, weight and color, were harvested at the mid of May of 2003, 2004 and 2005 in the full color stage and average weight of 224.3 gm and transported to the laboratory. The initial quality measurements were determined.

Fatty acids and alcohols composition of jojoba oil: The fatty acids and alcohols compositions of jojoba oil (unpublished data) were carried out as follows: the oil was firstly saponified and subsequently acidified, to liberate the free fatty acids, followed by methylation of the product [18]. The methylated product as well as the alcohols fraction was separately subjected to capillary GLC analysis.

Transformation of jojoba oil to trans (Isomerized) oil (TJO): Jojoba oil (Iodine value, 85; saponification value, 93 and acid value, 0.2) was obtained from Egyptian Company for natural oils. The cis unsaturated fatty acids of the oil were functionalized at the bond region to trans-fatty acids. This isomerization acquires the fatty acids solid waxy properties. Among the different methods published for isomerization, Arnon method [15] was selected for transformation of liquid jojoba oil into solid state as follows:

A solution of 500 gm oil in 500 ml petroleum ether (60-80°C) and 50 ml of 2 M $NaNO_2$ was heated until reflux and then 16 ml of 6 M HNO_3 was added by drops within 5 min after which heating was continued for 15-20 min. The hot solution was immediately transferred to a separatory funnel and was washed with hot water (50°C, 5×50 ml) until pH 7 was reached. The solvent was evaporated and the residue was left in a beaker to solidify. The melting point of the product was determined. The conversion of cis-fatty acids of Jojoba oil to trans-fatty acids was followed by Infra-red spectrophotometry (IR) since the trans fatty acids have a strong characteristic absorption in the infrared region of the spectrum at 10.36 microns, while the cis forms of these acids do not have a comparable absorption in this area [15]. The product was subjected to IR analysis to prove the transformation of cis double bonds to trans.

Fruit coating treatments: Coating of the selected Valencia orange fruits was carried out with TJO at the concentrations of (5, 10, 15, 20, 25 and 30%) admixed with Jojoba oil. Thus, the molten TJO and the hot JO (60°C) were mixed thoroughly before the coating process. The selected fruits, which randomly subjected to the different isomerized Jojoba wax, were hand coated (0.4 ml per fruit) at 25±1°C and subsequently air dried.

The coated orange fruits were compared with commercial export wax (E-wax) and also with uncoated fruits (control). Exported wax which used in citrus packinghouses was obtained from Egyptian company for mechanical and electrical industries. The composition of the Export wax in the form of water emulsion, posses 22% solids materials was contained (shellac, kalaphonia, polyethylene emulsifier and water).

Treated and untreated fruits put in carton boxes (6 kg in two layers of fruits), stored at 5°C±1 and relative humidity 85-90% for 60 days as simulation of export shipment and the initial quality measurements were determined. At 15 days intervals, fruit sample (15 fruits for each treatment) was removed from cold storage to determine fruit quality assessments and shelf life at 20°C and 55-60% RH was examined too.

Fruit quality assessments

Weight loss: Fruits were periodically weighed and the loss in mass weight was recorded for each replicate. Data were calculated as percentage.

Decay percent: Decayed fruits (physiological and microbial decay) were discarded in each sample and decay percent was recorded till the end of experiment.

Respiration rate: Individual fruits for each treatment were weighed and placed in 2-liter jars at 20°C. The jars were sealed for 3 h with a cap and a rubber septum. The resulting O_2 and CO_2 samples of the headspace were removed from the septum with a syringe and injected into Servomex Inst. Model 1450C (Food Pack Gas Analyzer) to measure oxygen and carbon dioxide production. Respiration rate was calculated as ml CO_2 kgG^{1} hG^{1} [19].

Soluble Solids Content (SSC): Individual Valencia orange fruits were ground in an electric juice extractor for freshly prepared juice. Soluble solids content was measured using a T/C hand refractometer Instrone (Model 10430 Brix-readings 0-30 ranges Bausch and Lomb Co. Calif., USA [20].

Titratable Acidity (TA): Total acidity (expressed as citric acid %) was determined by titrating 5-ml juice with 0.1N sodium hydroxide using phenolphthalein as indicator [20].

Ascorbic acid (Vitamin C): Ascorbic acid content was measured using 2, 5-6 dichlorophenol indophenols' method described by A.O.A.C. [20].

Experimental design and statistical analysis: The design for this experiment was a Completely Randomized Design (CRD) with three replications. Data were analyzed with the Analysis of Variance (ANOVA) procedure of MSTAT-C program. When significant differences were detected, treatment means were compared by LSD range test at the 5% level of probability in the three investigated seasons [21].

RESULTS

The transformation of jojoba oil to trans solid waxy material via isomerization by Arnon method [15] gave a good yield (40%) and the product has melting point of 45°C. The formation of trans double bonds was proved by IR spectroscopy (Fig. 1).

IR absorption of the product shows a strong absorption at 10.36 microns, which is characteristic to trans-isomers, indicating the transformation of cis-fatty acids into trans-fatty acids. The GLC analysis of original Jojoba oil (unpublished data) showed the presence of $C_{20:1}$, $C_{18:2}$ and $C_{18:1}$ (eicosaenoic acid, linoleic acid and oleic acid respectively) with long chain fatty alcohols (eicosenol, C_{20} and docosenol, C_{22}).

Fruit quality evaluation

Weight loss percent: Weight loss mainly consists of losses of water through transpiration and carbon gas exchange. Fruit weight loss was directly proportional to the storage period, as shown in (Table 1). The average weight loss percentage of Valencia orange significantly increased gradually with extending storage period up to 60 days at 5°C as well as after holding at 20°C for 7 days (shelf-life).

Although, Trans Jojoba Oil (TJO) treatments caused lower significant weight loss than control fruits, the percentage of weight loss decreased as the concentration of TJO increased (Table 1). After two months of storage at 5°C, the most pronounced loss (5.41%) was recorded in Valencia fruits coated with 30% TJO compared with uncoated fruits (control, 8.73%) and was significantly

Table 1: Weight loss percent of Valencia orange fruits coated after harvest with different concentrations of Trans Jojoba oil coatings (TJOCs) and stored at 5°C for 60 days and kept at 20°C for 7 days (shelf-life)

	4 weeks at 5°C		8 weeks at 5°C	
Trans Jojoba Oil Coatings (TJOCs)	At transfer	7 days at 20°C	At transfer	7 days at 20°C
TJO 5%	4.42b±0.042	0.71f±0.015	8.03b±0.070	1.33ij±0.017
TJO 10%	4.20c±0.038	0.65fg±0.023	7.64c±0.061	1.28ij±0.021
TJO 15%	4.13c±0.053	0.60fgh±0.015	7.09d±0.056	1.23jk±0.012
TJO 20%	4.07c±0.023	0.53gh±0.017	6.24e±0.046	1.15k±0.010
TJO 25%	3.71d±0.045	0.50h±0.012	5.83f±0.068	1.12k±0.017
TJO 30%	3.66d±0.066	0.47h±0.015	5.51h±0.061	1.08k±0.012
E-wax	3.61d±0.029	0.58fgh±0.023	5.58g±0.049	1.14k±0.010
Control	4.73a±0.147	0.89e±0.012	8.73a±0.066	1.38i±0.015
LSD at 0.05 of storage (S)	0.049		0.046	
LSD at 0.05 of Coatings (C)	0.098		0.091	
LSD at 0.05 (S×C)	0.139		0.129	

Data are means of three replicates of 5 fruits each. (Average of three seasons)

Table 2: Decay percentage of Valencia orange fruits coated after harvest with different concentrations of Trans Jojoba oil coatings (TJOCs) and stored at 5°C for 60 days and kept at 20°C for 7 days (shelf-life)

	4 weeks at 5°C		8 weeks at 5°C	
Trans Jojoba Oil Coatings (TJOCs)	At transfer	7 days at 20°C	At transfer	7 days at 20°C
TJO 5%	1.29i±0.017	1.79i±0.021	5.36b±0.044	7.58b±0.021
TJO 10%	1.13j±0.015	1.68j±0.027	4.88c±0.061	6.89c±0.020
TJO 15%	0.92k±0.017	1.44k±0.015	4.26d±0.040	5.98d±0.025
TJO 20%	0.84l±0.012	1.38l±0.017	4.03e±0.023	5.36e±0.020
TJO 25%	0.70m±0.015	1.26m±0.012	3.60f±0.052	5.14f±0.017
TJO 30%	0.66m±0.010	1.18n±0.015	3.49g±0.012	5.00g±0.015
E-wax	0.68m±0.021	1.20n±0.015	3.58f±0.015	5.02g±0.023
Control	1.41h±0.017	1.89h±0.023	5.73a±0.020	7.98a±0.021
LSD at 0.05 of Storage (S)	0.026		0.019	
LSD at 0.05 of Coatings (C)	0.053		0.037	
LSD at 0.05 of (S×C)	0.074		0.053	

Data are means of three replicates of 5 fruits each. (Average of three seasons)

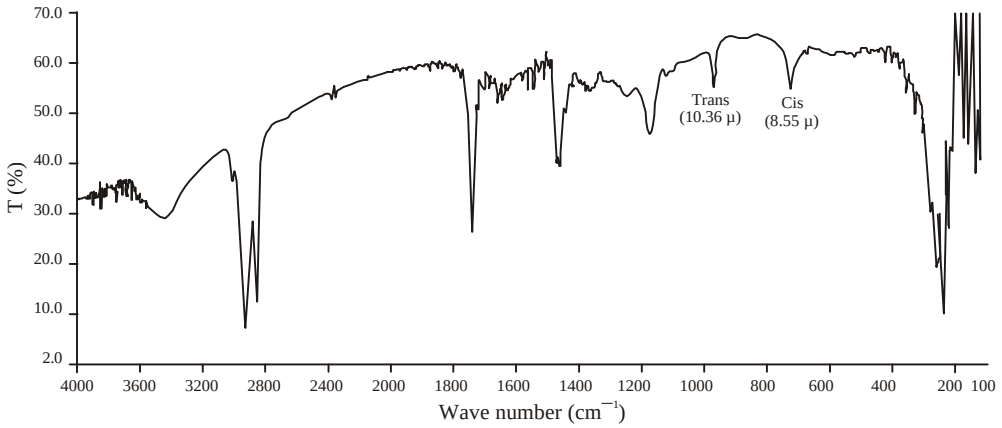

Fig. 1: Infra-red spectroscopy of isomerized jojoba oil

equal to fruits waxed with commercial export wax (5.58%). Further significant increase in weight loss percent was noticed after fruits kept at 20°C for one week (marketability).

It can be concluded that, the same trend of weight loss was obtained with approximately loss in weight by sixth percent than weight loss after cold storage whether at one or two months in all coated and control fruits.

Decay percent: Valencia orange fruits coated with all concentrations of TJO-coatings and also control fruits withstand free from pathogenic rots or microbial fruit deterioration during cold storage up to 60 days at 5°C. It can be stated that the average decay percent shown in (Table 2) recorded the percentage of softening mere incidence or breakdown, either during storage or marketing life at 20°C for one week. Table 2 revealed that decay percent in Valencia orange, as softening symptoms, significantly increased as TJO-coatings concentrations decreased during cold storage at 5°C as well as after shelf-life at 20°C for one week.

Fruits waxed with TJO at 30% had the least deterioration percent (o.66 and 3.49%) followed with that treated with Export wax (E wax) and 25% TJO (0.68, 3.58 and 0.70, 3.60%) respectively, compared with unwaxed fruit (control) showed the highest percent (1.41and 5.73%) after the two periods of cold storage (4 and 8 weeks) throughout the successive seasons of study.

On the other side, shelf-life as marketability indicator was inspected after kept Valencia orange fruit for 7 days at 20°C after removal from cold storage. It can be concluded that, the same trend of decay percentage was found significant in all TJO treatments and control ones. Meanwhile, it can be concluded that the softening incidence through shelf-life period increased by 1.4% than decay percent during cold storage either one nor two months in all coated and control fruits.

Respiration rate: It can be seen from (Fig. 2) that there was a noticeable significant increase in respiration rate of coated Valencia orange fruits reaching a peak value after 4 weeks. Subsequently, the decrease after 8 weeks of cold

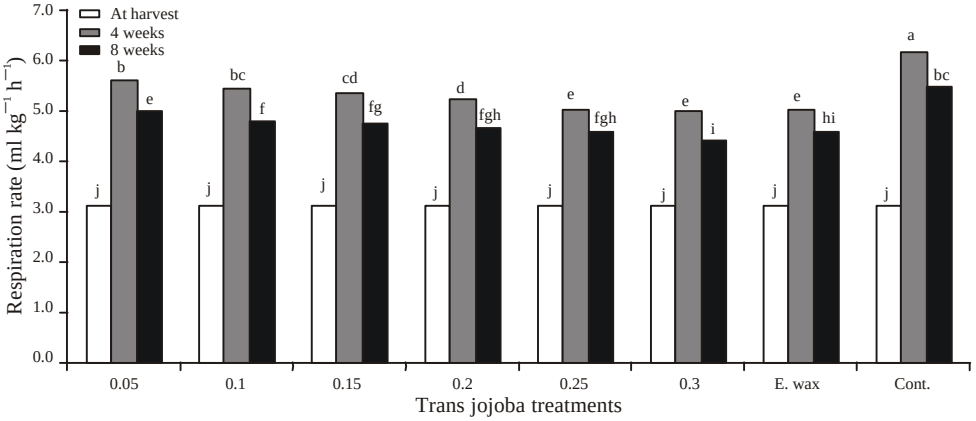

Fig. 2: Respiration rate of valencia orange fruits coated after harvest with different concentrations of Trans Jojoba Oil (TJOCs) and stored at 5°C for 60 days. Values are the means of 3 replicates of 5 fruits each. The letters represents LSD at 0.05 level

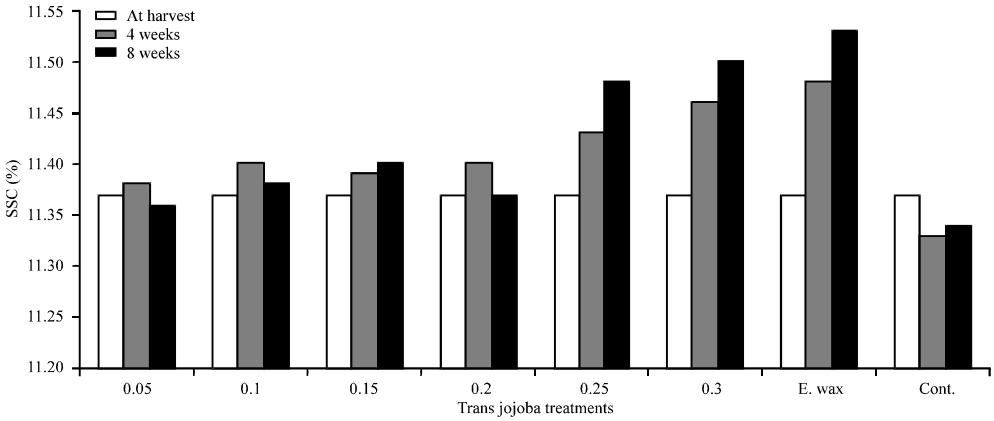

Fig. 3: Soluble solids content (SSC) of valencia orange fruits coated after harvest with different concentrations of Trans Jojoba Oil (TJOCs) and stored at 5°C for 60 days. Values are the means of 3 replicates of 5 fruits each. The letters represents LSD at 0.05 level

storage at 5°C was slight during the three seasons of investigation. The high coating concentrations of TJO tended to have the effective role in reducing the rate of respiration of orange fruits than control ones as well as in export wax treatment.

Although, fruits waxed with 25% TJO showed equal respiration rate with export wax treatment (5.03, 4.58 and 5.03, 4.55 ml kg^{-1} h^{-1}) respectively. The Trans Jojoba Oil treatment at 30% showed the least rate of respiration (4.98, 4.43 ml kg^{-1}hr^{-1}) after one and two months respectively. Control fruits had the highest respiration rate (6.17 and 5.51 ml kg^{-1} h^{-1}) compared with the initial value of (3.11 ml kg^{-1} h^{-1}) at harvest.

These results indicated that beneficial effect of TJO treatments on the reduction of respiration rate was maintaining Valencia orange fruit quality and expanding their storage duration and marketable life.

Soluble Solids Content (SSC %): According to Fig. 3, it is clear that soluble solids content (SSC) of Valencia orange fruit was not affected significantly either by Trans Jojoba Oil coatings or cold storage duration. However, it was noticed that, there was no clear change in SSC content in isomerized jojoba treated fruits throughout the period of cold storage at 5°C, in comparison with the initial SSC value at harvest (11.37%).

At the end of the storage, the highest soluble solids content (11.53%) was obtained in fruits coated with exported wax (E wax), followed with fruits coated with 30 and 25% TJO which exhibited equal SSC values (11.50 and

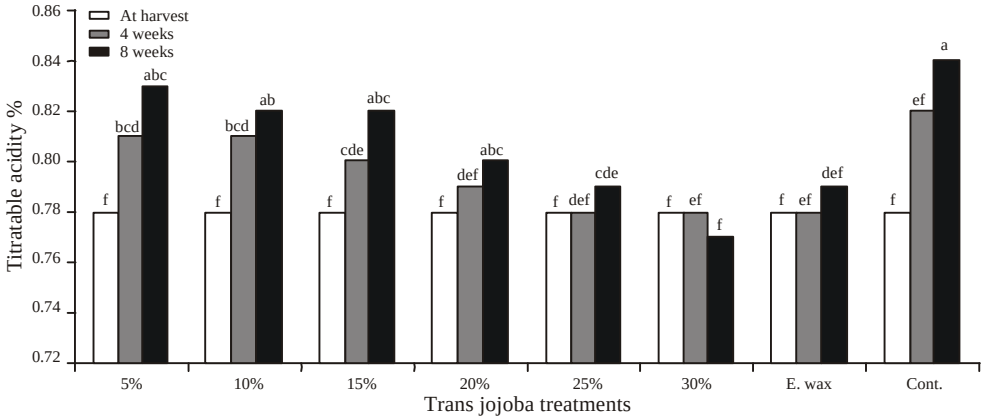

Fig. 4: Titratable acidity (as citric acid%) of valencia orange fruits coated after harvest with different concentrations of Trans Jojoba Oil (TJOCs) and stored at 5°C for 60 days. Values are the means of 3 replicates of 5 fruits each. The letters represents LSD at 0.05 level

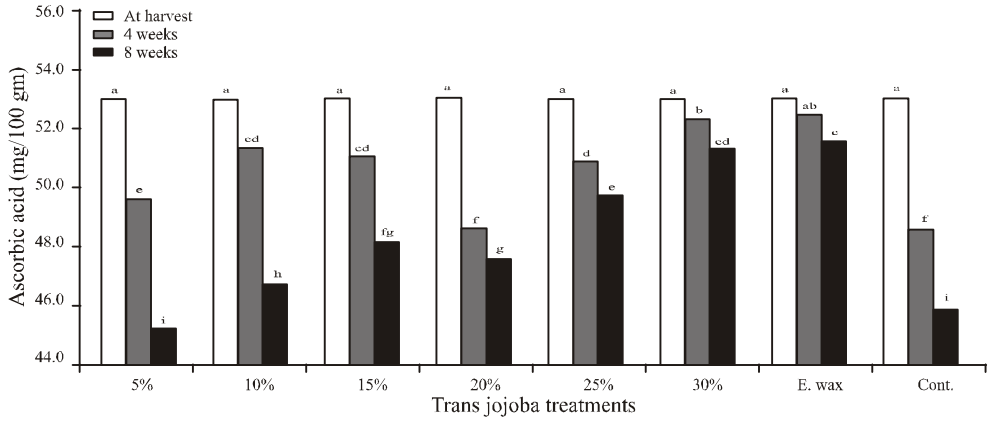

Fig. 5: Ascorbic acid content of valencia orange fruits coated after harvest with different concentrations of Trans Jojoba Oil (TJOCs) and stored at 5°C for 60 days. Values are the means of 3 replicates of 5 fruits each. The letters represents LSD at 0.05 level

11.48%) respectively. Whereas, control fruits had the least significant value (11.34%) with respect to all treatments under investigation.

Titratable Acidity (TA %): Concerning the changes of titratable acidity (TA) of Valencia orange fruits due to the coatings of TJO and cold storage treatments, (Fig. 4), there was a slight significant increase in fruit acid content as the storage period progresses, however, it decreased with increasing Trans Jojoba Oil concentrations.

At the end of the storage period, control fruits showed the highest significant titratable acidity content (0.84%) compared with its initial value at harvest (0.78%) The lowest acid value (0.77%) was recorded in fruits previously waxed with 30% TJO. Meanwhile, both Exported wax (E wax) and 25% TJO coated fruits had equal acid content (0.79%) at the 8th week storage at 5°C of the three successive seasons.

Ascorbic acid content: Ascorbic acid content (Vitamin C) of Valencia orange fruit showed a progressive and significant decrease due to coatings with isomerized Jojoba wax under cold storage period including control fruits (Fig. 5).

After 8 weeks of cold storage at 5°C, it is remarkable to conclude that, uncoated fruits (control) lost 13.50% of its vitamin C content compared with 3.35% in the content of orange fruit coated with 30% Trans Jojoba Oil. The same trend was observed at shelf-life period, when treated orange fruits are transferred from cold storage at

5°C and kept at 20°C for one week. The differences between treatments, storage period and their interaction were significant.

DISCUSSION

In the present study, the first applications of Trans Jojoba Oil (TJO) coatings, maintained the quality of Valencia orange fruits up to 8 weeks storage at 5°C. Respiration rate were indicative of significant increase rate till the peak value (4 weeks), followed by reduction trend during the second month of cold storage relative to TJO treatments. The Application of Jojoba-based waxes significantly reduced internal O_2 levels and increased internal CO_2 and were consistent with the reports of Hagenmaier and baker [7], Ergun *et al.* [9] and Petracek *et al.* [22]. They found that there was no relationship between the gas permeability of the coatings and the concentration of oxygen and carbon dioxide inside the fruits. These findings indicate that the O_2 and CO_2 gases do not leave the fruits according to their permeability values but that there is apparently another pathway through which the passages of the gases take place. Moreover, Mannheim and Soffer [5] and Hagenmaier and Shaw [14] claim that there are two pathways for gas exchange: (1) the coatings forms an additional barrier on the peel through which the gas must be permit or (2) the coatings plug openings in the peel [5]. In addition, Saftner *et al.* [23] explain these findings that the coatings were additionally inhibiting the out migration of ethylene and possibly other volatile from the fruit. During storage at 20°C, fruits produced high amounts of CO_2 but with lower values in TJO coating treatments than uncoated fruits which are inconformity with that reported by Ergun *et al.* [9] and Petracek *et al.* [22].

Regarding the effectiveness of TJO treatments in reducing fresh weight loss percent and maintaining good visual appearance in Valencia orange fruits (either at storage at 5°C or marketable period at 20°C), were similar (Table 1). This was reported for citrus fruits by Hagenmaier and Shaw [2], Ergun *et al.* [9], Saftner *et al.* [23]and Parat *et al.* [24] on mamy sapote fruits and 'Gala' apples. In almost all cases, waxed commodities lost weight more slowly than unwaxed controls. In fact, weight reduction has been recommended as a criterion of good waxing [14]. However, it would go too far to recommend by Hagenmaier and Shaw [14] that the permeability for citrus coatings, as barriers should be low for water vapor to reduce transpiration as much as possible. Moreover, the use of wax-based coatings treatment in refrigerated air storage, as potential alternatives to CA storage, offered a potential extension of storage life in apples [23] and Grapefruit [11, 22] over air storage. In contrast to gas exchange, the resistance of peel to water vapor transport is more dependent on the coating thickness than on the type of coatings [5].

Decay was mentioned as one of the limiting factors for postharvest life of citrus fruit because of removing the natural wax of citrus peel through the handling in packinghouses [6, 22]. There are two major problems limit the long-term storage capability of citrus fruits: the first: is pathological breakdown, leading to decay; the second: is physiological breakdown, resulting in the appearance of the various rind disorders [24]. In this study, Valencia orange fruits coated with different concentrations of TJO could be stored for 8 weeks at 5°C without pathological breakdown (rots) as shown in (Table 2). While, physiological breakdown as softening appearance ranged between 5.36-3.49% with directly proportional to TJO concentrations. These results of TJO treatments confirmed the previous findings of Huating [6] who observed that the Chilling Injury (CI) percentage score higher than 5% will indicate that the fruit's appearance is damaged and thus has an impact on the consumer's purchasing decision. Moreover, Petracek *et al.* [22] suggested that postharvest pitting as physiological disorder can be controlled by improving the gas permeability of applied wax citrus peel barrier. In addition, similar significant trend of decay percentage at 20°C (shelf or marketable life) were noticed in TJO-coated fruits. While, the softening incidence during shelf-life period increased by approximately 1.4% than decay percent through cold storage at 5°C for one or two months (Table 2). Also Huating [6] and Ergun *et al.* [9] suggested that marketable life was extended of waxed fruits due to the appearance of decay and slight fungal decay on fruit surfaces in former treatments compared with unwaxed fruits.

Upon soluble solids (SSC) and titratable acid (TA) content, were not significantly affected either by coatings or cold storage duration (Fig. 3 and 4), which are similar to that reported byBalduin *et al.* [8], Ergun *et al.* [9] and Landaniya and Sonkar [17] on mandarin, mamy sapote fruits and mango, respectively. Bums and Echeverria [25] reported that wax application had no effect on acidity (%) and brix level of stored 'Valencia' fruits. Although TA showed a slight increase throughout storage period, the highest TJO concentration (30%) had the lowest acid value relative to waxed and control fruits.

In all cases investigated, a significant decrease of vitamin C could be observed in Valencia orange fruit between the initial value and the Vitamin C content at the

end of storage period. Mahrouz *et al.* [26] had the same findings in the waxing treatments of Clementine citrus fruits.

CONCLUSIONS

It can be concluded that, the first utilization of Trans Jojoba Oil (TJO) as Valencia orange fruit coating is promising wax than the other investigated coating materials, especially in the range of 20-30% concentrations. It can be predicted that TJO coating's resistance to gas exchange is strongly influenced its ability either by blocking pores on the surface of the fruit or, acting as barriers not only to gases migration to restrict respiration, but also to water vapor transfer reducing transpiration and weight loss. Finally, TJO wax can use successfully without any additives as coating product from natural source. It is substitute or alternative to commercial wax used in handling citrus fruit for export. Valencia orange fruit coated with TJO stored in a good quality up to 8 weeks, which was enough period for sea or land shipment for exported citrus fruit.

ACKNOLEDGEMENTS

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Characteristics of Smallholder Pig Production in Southern Kaduna Area of Kaduna State, Nigeria

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Abstract: This paper presents the results of a survey of pig producers in Southern Kaduna Area of Kaduna State, Nigeria. A total sample of 300 respondents spread over 2 LGAs and 5 village areas was drawn. Only households which kept pigs at the time of the study were sampled. Data were collected from the respondents through the use of structured questionnaire. Data generated included demographic characteristics of pig owners, pig distribution by household, management practices and problems, sources of feed by season, type of housing and livestock disease profile. The data collected were analyzed using descriptive statistics such as means, ranges and percentages. The results show the influence of some socio-economic variables on pig production. Over 85% of the respondents acquired their foundation stock from the open market, while others came from neighbours' herds. Most respondents expressed the desire to increase their holdings. However, feeding was most frequently mentioned as a limiting factor in increasing pig herd size. Brewers' residues (*Burukutu* wastes) and household wastes were the major sources of animal feed. There was a very low awareness level of the use of commercial feeds. Only 10% of the respondents indicated having used any form of commercial feed. Implications for improved pig production in Nigeria's sub-humid zone are drawn.

Key words: Pigs % smallholder % management % Kaduna State % Nigeria

INTRODUCTION

Nigeria, like many other developing countries, is facing the problem of shortage of dietary animal protein. The gravity of this problem is increasing with the growing population and urbanization. In Nigeria, the daily animal protein intake is below the recommended minimum level of 65 gm per caput per day. It has been observed that only 8.4 gm of the 53.8 gm of protein consumption level of Nigerians is derived from animal sources [1] suggesting less than 16% contribution of animal products to protein consumption of Nigerians. This is very poor indeed when compared with countries like USA with about 69% of total protein being derived from animal sources [2]. Due to the acute shortage of animal protein in the diet of the average Nigerian, there is the need to increase the production of domestic animals, which are conventional sources of animal protein.

Sources of animal protein in Nigeria include beef, milk, pork, poultry, sheep, goats, fish and game animals. Of these sources, pork represents one of the fastest ways of increasing animal protein, since pigs grow at a faster rate and are more prolific than cattle, sheep and goats [3, 4]. Also, pigs excel other red meat animals in converting feed to flesh [5]. The importance of pigs in the livestock industry in Nigeria cannot be over-emphasized. Although, pigs represent about 4% of the total domestic livestock in Nigeria [6], they display a unique ability to adapt and survive in areas where they are found. The pig is a potential protein deficit gap-filler considering the population in Southern Kaduna area of Kaduna State and the generational interval among other favourable attributes of the animal.

The pig is not only a source of protein; it serves as an investment alternative and source of additional income especially among the women [7]. The rearing of pigs is usually an individual concern and herds are not usually found in commercial quantities. Pig keeping is a secondary enterprise and represents some proportion of the income earned by women. Incomes derived from the sale of pigs are usually spent on acquisition of household goods and in meeting social and cultural obligations.

Of the three major production systems recognizable in Nigeria, the semi-intensive system of pig production is

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the most prevalent in areas with high population density like the Southern Kaduna [8]. This production system has a low labour input and low priority adjunct to the intensive management system.

The predominant breeds of pigs kept in the study area are the Large White and crosses between Large White, Hampshire and Duroc. The existence of a large number of exotic breeds especially the Large White and their crosses could be attributed to the maintenance of pure exotic breeds like the Large White and Hampshire multiplied by Ahmadu Bello University Farm, Zaria [8].

Southern Kaduna area is well known for the abundance of pigs in its villages. Pigs are quite popular as food but the economic basis of village production is sales to traders from the Southern part of Nigeria and the West African sub-region. Kafanchan pig market in Southern Kaduna area is well noted for this [9].

Under the semi-intensive system of production, animals are partly confined and allowed to scavenge on kitchen wastes with occasional supplements of maize by-products or local beer residue (Burukutu wastes). Cereal brans and fresh grasses are also fed to the pigs. This is usually not supplemented with any extra protein source. Feeds are generally bulky and of low nutritional quality. Feeding in some cases is done only once a day with little or no extra clean water supplied to the pigs besides that used to mix the feed.

Labour requirements, however, often limits the number of animals kept at any given time period under this management system. Due to high population densities and land squeeze most especially during the rainy season, animals are frequently confined. The growing human population in Nigeria generally, coupled with rapid urbanization and the subsequent pressure on land will compel further confinement which will directly necessitate intensive management practices. There is therefore, the need to develop appropriate feeding strategies in consonance with perceived shortage (costly nature) of conventional feeds. This situation can effectively limit the size of rural pig holdings. The unique position of agro-industrial by-products in pig feeding system underscores the need for research to fully exploit these feed resources. These agro-industrial by-products such as brewers' spent grains are readily available and relatively cheap. As monogastric livestock, pigs cannot depend entirely on natural vegetation and thus the question of feed takes on greater importance than for the ruminants.

This study reports part of a larger survey of pigs production and marketing in Southern Kaduna area of Kaduna State, Nigeria which investigated the socio-economic factors of producers and their influence on pig management practices and also the economics of pig production and marketing.

Objectives of the study: The general objective of the research was to study the management practices and problems associated with small-holder pig production in Southern Kaduna area of Kaduna State, Nigeria with the aim of better understanding the prevailing production systems.

The specific objectives include the following:

- To describe the system and structure of pig management in the study area;
- To describe the attitude of pig producers to important management practices as they relate to pig production; and
- To draw implications for enhancing pig research, development and extension services in the study area in particular and Nigeria at large.

MATERIALS AND METHODS

The study area: The study area is Southern Kaduna area of Kaduna State, Nigeria. The state was chosen for the study primarily because it ranks among the highest pig population area in Nigeria such as Benue (703,438 pigs), Plateau (535,319), Gongola (476,143) and Ondo (291,304). The pig population of Kaduna State is 249,651 representing about 7.3% of the total pig population in Nigeria [6]. Furthermore, Kaduna State has the highest number of pigs per household [6].

The state is situated between latitude 09°30'N and longitude 08°30'E in the Northern Guinea Savannah. The rainy days last between 190 to 200 days with distinctive dry and rainy seasons. The study location has two distinctive seasons; a dry season (November-April) and a rainy season (May-October).

The study was conducted in two Local Government Areas (LGAs) of Kaduna State: Jama'a and Zango-Kataf LGAs. The study area is bounded in the North by both Kajuru and Kauru LGAs, in the east by Lere and Kaura LGAs, in the West by both Kachia and Jaba LGAs and in the south by Akwanga LGA of Nassarawa State (Fig. 1).

The soil is rich and suitable for the cultivation of a wide range of crops. Most of the ethnic groups are farmers that keep a good number of pigs, small ruminants and poultry in addition to arable cropping.

Southern Kaduna is sub-humid and is predominantly Christian area of Kaduna State. The location was

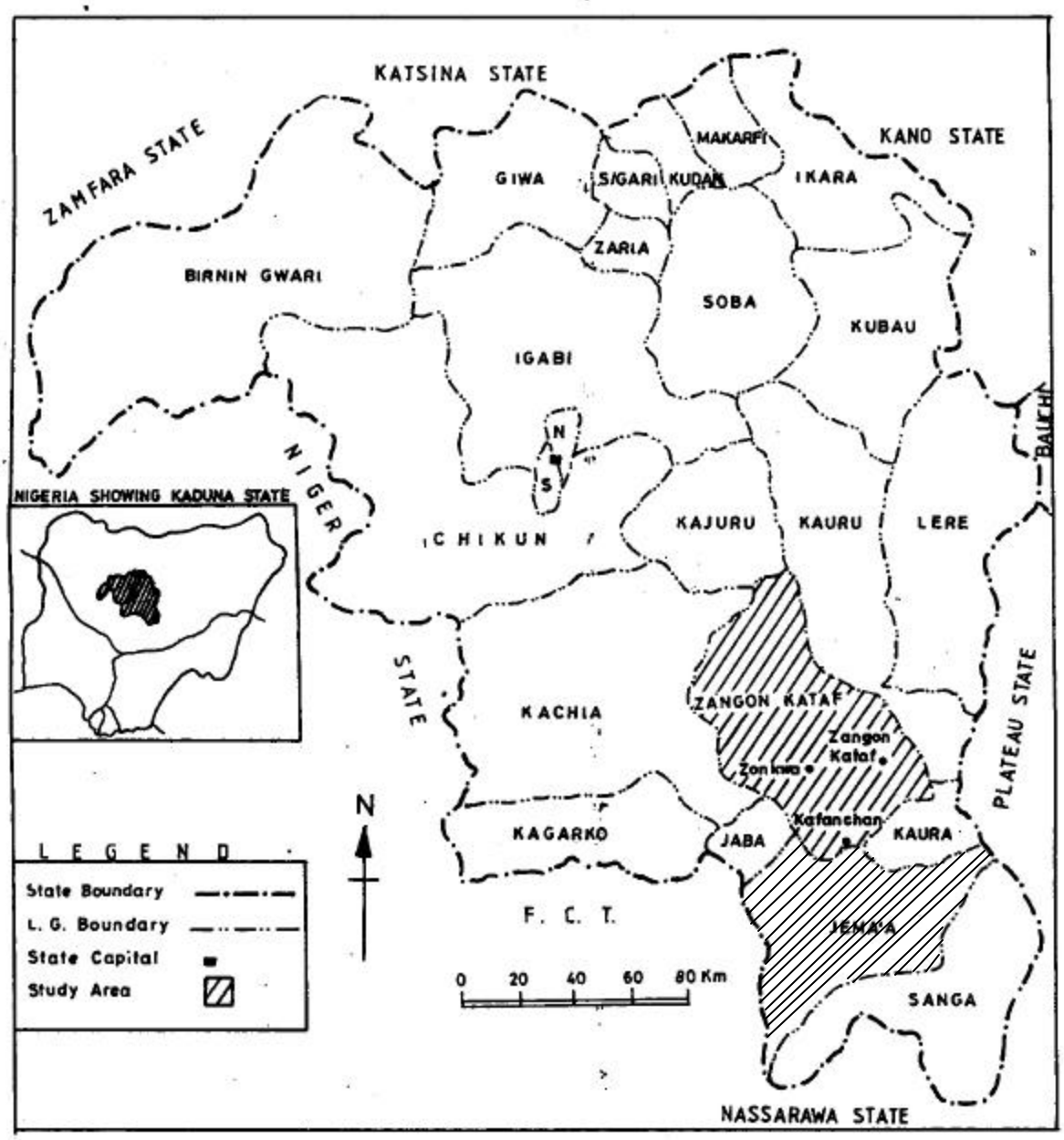

Fig. 1: Kaduna State showing the study area

specifically chosen for the study because majority of the farmers in the area are involved in pig production. In Jama'a LGA alone, out of the total of 2,368 farm families identified, 1,804 representing 75% of the farm families, rear pigs [10]. Secondly, the area is a known potential pig market in the country [9].

Data collection: The Southern Kaduna area of the State was chosen for the study. Total samples of 300 households were sampled from two Local Government Areas (Jama'a and Zango-Kataf LGAs). One hundred and fifty households were randomly drawn from each LGA. Within an LGA, five village areas were selected with at least, thirty households interviewed in each village area. The sampled population comprised households which kept pigs at the time of the study. The survey was conducted between June 2001 and May 2002 in order to cover both the dry and wet seasons.

Variables investigated include demographic characteristics of pig owners, pig distribution by households, management practices and problems associated with current management systems, others are sources of feed by season, preferred feedstuff and reasons for choice of feed materials and livestock disease profile.

RESULTS AND DISCUSSION

Demographic characteristics of pig farmers: Analysis in Table 1 shows that age affects production of pigs, since the lowest age group of 21-30 years has a mean pig herd size of 3.02, while the group above 60 years has 2.52. This is corroborated by the fact that there is a significant difference in herd size across age groups.

From the analysis, it can be adduced that the number of pig farmers of 87 within age bracket of 21-30 years is three times those in age bracket of 60 and above with a frequency of 29. The reason for this could be because the farmers within age bracket (21-30 years) are young and can easily bear the risk of accepting new innovations aimed at improving pig production. The fact that they are young shows they can still face the challenges of pig rearing given the demand of integrating both crop and livestock enterprises especially for labour.

Household size includes members from the age of seven years in a family that were engaged in productive activities. Table 2 shows that 37% of the respondents fell within the range of 1-5 members per household, while 40, 15 and 8% fell within the range of 6-10, 11-15 and 16-20 members respectively. The result shows that the larger the household size, the more the herd size of pigs, up to 11-15 household size.

Table 1: Age distribution of pig farmers by mean herd size

Age bracket (years)	Frequency of respondents	% of respondents	Mean herd size
21-30	87	29.0	3.02
31-40	79	26.3	2.97
41-50	60	20.0	2.84
51-60	45	15.0	2.66
Above 60	29	9.7	2.52
Total	300	100.0	

Source: Field work, 2002

Table 2: Distribution of pig producers by household size and herd size

Household size	Frequency of respondents	% of respondents	Mean herd size
1-5	111	37	2.87
6-10	120	40	2.84
11-15	45	15	4.15
16-20	24	8	3.48
Total	300	100	

Source: Field work, 2002

Table 3: Distribution of pig farmers by herd size

Herd size	Frequency of respondents	% of respondents
1-3	156	52
4-6	87	29
7-9	57	19
Total	300	100

Source: Field work, 2002

Analysis revealed that majority of the respondents (52%) had less than four adult pigs while 29% had between 4 and 6 pigs (Table 3). The mean adult herd size was 2.9±1.6. All the respondents were small-scale farmers. This conforms to an earlier report by Pathiraja *et al*. [11], that pig farming in Southern Zaria (now Southern Kaduna) is primarily a smallholder concern.

Table 4 shows that about 85% of the respondents acquired their foundation stock from the market, while others came from neighbours' herds. It was observed that the best breeding stock rarely goes to the market resulting in the use of foundation stock with poor breeding qualities. The results also indicate non-utilization of improved breeds from government farms, increased use of own stock and that of neighbours; this gives rise to inbreeding and consequently low productivity.

Table 4: Sources of parent stock

Source	Number	Percent*
Market	254	84.7
Inherited	20	6.7
Neighbour	96	32.0
Bred	79	26.3
Government farm	9	3.0
Borrowed	25	8.3
Others	10	3.3

*Total observations>100% due to multiple responses, Source: Field work, 2002

Table 5: Desire/plan to increase herd size

Decision	Number	Percent
Yes	193	64.3
No	18	6.0
Not decided	89	29.7
Total	300	100.0

Source: Field work, 2002

Table 6: Anticipated problems if herd size is increased

Problem	Number	Percent*
Feed	216	72.0
Health	170	56.7
Theft	67	22.3
Accident	48	16.0
Labour	144	48.0
Housing	196	65.3
Others	5	1.7

*Table observations>100% due to multiple responses, Source: Field work, 2002

Table 7: Sources of animal feed by season

	Dry season		Wet season	
Source	No.	%*	No.	%*
Household wastes	238	79.3	204	68.0
Brewers' residue (Burukutu waste)	204	68.0	186	62.0
Cut grasses	42	14.0	51	17.0
Commercial feeds	30	10.0	30	10.0
Animal fend for self	156	52.0	117	39.0
Don't know	9	3.0	6	2.0

*Total observations>100% due to multiple responses, Source: Field work, 2002

Most respondents expressed the desire to increase their holdings (Table 5). In the event that herd sizes are increased, however, feeding was most frequently mentioned as a limiting factor in coping with pig keeping (Table 6). This requires additional labour input in providing the feeds. It was observed that feeding, housing and health constituted the major constraints to increased pig production in the study area. Housing, health and nutrition are interrelated. Inadequate housing can predispose pigs to diseases and possibly trigger-off (facilitate) the spread of contagious diseases especially where animals have been over-crowded in a place.

Household wastes and brewers' residues (burukutu wastes) were the major sources of animal feed (Table 7). Due to the fact that most respondents practiced the semi-intensive system of management whereby animals are partially confined most especially during the dry season and totally confined during the wet season, cut grasses are fed along with household wastes such as peels of yams, potatoes, cocoyam; brans and cereal crops like maize, sorghum and millet. Only 10% of the respondents indicated having used any form of commercial feed. Closely associated with sources of feed was the prevailing management system practiced. The fact that almost 58% of the respondents confined their animals year round and another 42% confined seasonally justifies the availability of other sources of feeds such as the high incidence of brewers' residues (burukutu wastes) plus household wastes. Another reason may be the fact that most of the respondents are burukutu brewers (local beer brewers) and crop farmers. A large proportion of cereal and root crop residues which are available from households and unsuitable for marketing and family use, are used as pig feeds.

All the respondents provided some form of housing to pigs. Various types of housing materials were used by the respondents depending on their scale of production (size of holding). Three types of pig houses were identified in the study area: (i) the mud-brick walls with thatched roof and rammed earth floor type, (ii) the cement-brick walls with zinc roof and concrete floor type and (iii) the burnt-brick walls with zinc roof and concrete floor type. Majority of the respondents had low cost pig houses built mainly from locally available materials and household labour. Majority (62%) of the respondents used mud-brick walls with thatched roof type of housing while 30% of the respondents used the cement brick walls with zinc roof and the remaining 8% used the burnt brick walls with zinc roof and concrete floor. Although most of the respondents kept their pigs in the mud-brick walls with thatched roof and rammed earth floor because of its cheapness. Apart from the undurability of the building, it predisposes the pigs to diseases. The cement block walls with zinc roof and concrete floor had been advocated because of its durability and high level of hygiene. Apart from durability and hygiene, the

Table 8: Prevalent diseases in the study area

	Dry season		Wet season	
Diseases	No.	%*	No.	%*
Diarrhoea (gastroenteritis)	208	69.3	133	44.3
Cough/pneumonia	44	14.6	71	23.7
Mange (itching/lousing)	195	65.0	106	35.3
Anaemia	64	21.3	82	27.3
Ascaris suum	30	10.0	17	5.7
Worms	154	51.3	182	60.7
Mastitis	40	13.3	31	10.3

*Total observations>100% due to multiple responses, Source: Field work, 2002

economy of better housing would favour such housing in the long run [12].

Survey results indicated that the main disease problems reported by the respondents were helminthoses, cough, diarrhoea, skin conditions mainly sarcoptic mange (itching/lousiness) and the presence of Ascaris suum in pig faeces. Mange prevalence in herd was about 68%. There was also high incidence of cough and diarrhoea. Cough prevalence was higher during the dry season from December to May while diarrhoea was more prevalent from July to September corresponding to the peak of the wet season. Helminthoses, sarcoptic mange and gastroenteritis were observed to be the commonest diseases in the herd (Table 8) followed by anaemia, pneumonia and mastitis (24.3%, 19.2% and 11.8% respectively).

Mange (louse infestation) can cause considerable production losses because of the biting nuisance, especially when prevalence is as high as 65% during the dry season as recorded in this study. The low incidence observed in the wet season is due to high humidity during the rainy season which is not conducive to lice development.

Although, there was high incidence of diarrhoea in the study area, respondents noted that it was more common with young pigs and was observed to be probably responsible for their mortalities. Most mortalities have been reported to occur in piglets of the pre-weaning age of three months and below. The most important cause of mortality was observed to be poor mothering ability (crushing or eating of the young) due to poor housing and inadequate nutrition, followed by diarrhoea.

Over 60% of the respondents vaccinated their pigs against diseases. It was equally discovered that 35% of these respondents used modern method of vaccination while 25% used local or ethno-veterinary vaccination practices. The materials used as local means of treatment in the study area included iron-rich soil and wood-ash in combating anaemia (shortage of iron in the blood) in piglets. Although wood-ash will not provide iron; but it provides other important materials such as calcium and phosphorus which are important for the growth of the piglets' bones.

For mange, treatment involves the removal of scales and dirts through washing with soap, scrubbing with brush and smearing with oil. For the control of ectoparasites also, hot wood ash, corn shaft and lime are used.

Response to disease attack varied among the respondents. Ten percent of the respondents slaughtered and ate pigs showing signs of disease attack, 15% did nothing, 35% gave local remedy while 40% called veterinarians to treat their pigs.

Generally, pig production in Kaduna State was a part-time occupation as a combination of animal species (domestic fowls, ducks, goats and sheep) were kept by the rearers, 78% of whom were also crop farmers. About 27% of the respondents reared the pigs for both income generation and consumption while the remaining 63% of the respondents kept pigs mainly for commercial purposes. Pigs were sold at different ages or sizes among the respondents. Ten percent of the respondents sold their pigs at the age of 6-12 months. About 7% respondents sold them at less than 6 months of age, 60% sell theirs at the age of 1-2 years, while 23% sell at less than 6 months of age. This was supported by Bawa *et al*. [12] that pigs can be reared and marketed at different weight and ages. The selling price depended on the age and weight of the pigs at the time of sale.

On access to credit, 60% of the respondents belonged to one form of cooperative group or another while the remaining 40% did not. Sixty six percent of the respondents indicated their willingness to obtain credit, 23% of the respondents were unwilling to obtain credit because they did not want to incur debts. Also 11% of the respondents were unwilling because of the problems of high interest rates on loans.

CONCLUSIONS

From the study, it is very clear that small-holder pig production has great potential in bridging the animal protein supply gap and also enhance the employment status of the farmers and the rural economy in general.

Disease control programmes, if carefully planned and executed should compliment adequate feeding and reduce

mortalities and increase overall productivity of pigs. The implications of higher animal productivity at the farmers' level can be seen in enhanced income and improved living standards of the farmers and their households as well as increased animal-source protein for Nigerians.

It is envisaged that a reduction in pig mortality especially under traditional management will go a long way in making more animal protein available to the general populace.

Evaluation of traditional remedies on a research station (National Veterinary Research Institute, Vom) to determine their effectiveness in controlling diseases and parasites.

The quality of feed given to pigs should be improved. The farmers should be educated through the use of extension workers, on feeding methods using non-conventional feeds and the need of the inclusion of protein mineral and vitamin in the diet of pigs.

Soft loans with adequate moratorium period could be given to pig farmers, this will aid expansion of the enterprise.

Without prejudice to the aforementioned, improved nutrition should be seen as the bottom-line to any successful programme of improved and increased pig production in Nigeria.

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Granitic Derived Soils in Humid Forest of Southwesthern Nigeria-Genesis, Classification and Sustainable Management

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Abstract: The genesis of four granitic derived soils in Ado-Ekiti, humid forest of Southwesthern Nigeria was studied. The soils have thick darkbrown (7.5YR4/3-10YR 4/3) coarse sandyloam/loamy sandy surface horizon, grading loan in the subsoils. The solum is strongly acidic (4.90) to moderately acidic (5.89), low in organic carbon, exchangeable bases and cation exchange capacity. Dominant pedogenic process which influence the rate of soil development in the area include hydrolytic weathering, which has resulted in ferrallitic pedogenesis. Others are lessivation pedoturbation, braunification, induration and colluvial deposition. Pedon A have been classified as Typic Udipsamment (ferralic Arenosol-Apomu series) Pedon B as Typic Plinthudult (Dystic Plinthosol-Iregun series), Pedon C as Typic Dystrudept (Ferralic cambisol-Balogun Series) and Pedon D as Plinthic Hapludox (Plinthic Ferralsol-Apomu series). Finally, recommendations for the sustainable use of these soils are discussed.

Key words: Granitic soils % genesis % classification % sustainable management % Southwestern Nigeria

INTRODUCTION

The relationship between landscape position and hydrological and geomorphic process has been well established for many landscapes [1-3]. Previous efforts at understanding the genesis, chemical and mineralogical properties of soils formed in the Basement complex of Southwestern Nigeria have been directed at the study of a veneer of pedisediment over saprolite [4, 5] suggested that a consideration of the interaction of episodic landscape erosion and pedogenesis was required to explain soil genesis. Earlier, a concept of cylic soil-landscape development which permits the recognition of lithologic discountinuities [6] was proposed for the genetic interpretation while [7] suggested ferralitic pedogenesis.

In the basement complex area there is a strong relationship between topographic position and soil genesis [8]. The difference in the rate of hydrologic and geomorphic process in various landform positions causes differences in the types and intensity of pedogenic processes and typically result in a non-random distribution of soil taxi and properties in a landscape.

Landscapes position influences runoff, drainage, soil erosion, hence soil genesis is affected. Different soil properties encountered along landscapes will affect pattern of plant production, soil physical properties such as clay content distribution with depth, sand content and pH have been shown to be highly correlated with soil genesis [9] while soil organic matter has been shown to vary with slope position [10].

Amusan and Ashaye [4] suggested that the genesis of soil begins with the physical and chemical alteration of minerals at the rock-saprolite interface. This study therefore examines the genesis of soils resulting from in-situ weathering of granitic parent material under humid tropical climate and attempts at classifying the soil and recommending appropriate sustainable management systems for the soils.

MATERIALS AND METHODS

Description of the study area: The location lies between latitude 7°31'N and 7°49'N and longitude 5°3'N and 5°2'N in Ado-Ekiti within the humid forest Southwestern Nigeria. The area is characterized with distinct dry (November to early March) and wet (March-October) seasons. Rainfall pattern is bimodal with peak periods in June and September, mean annual rainfall is about 1367mm and average number of raining days is about 112 per annum. Temperature in this area is almost uniform throughout the year with very little deviations from the mean annual temperature of 27°C, February and March are the hottest months with mean temperature of 28 and 29°C

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respectively. The total number of sunshine h is about 5 h and mean annual radiation about 130 kcal/cm^3/year. The topography of the site is highly moderately steep slope with the highest point having slope not greater than 10%. The geology of the area is dominated by crystalline rock, which form part of the basement complex of southwestern Nigeria. The soils are mostly of granitic parent material.

The main vegetation of the area are trees, grasses, ferns, bush regrowth etc. some parts of the area have been put to cultivation of arable crops like maize and tubers such as Yam and Cassava while the remaining parts are allowed to fallow.

Field studies: The whole area was mapped using the rigid grid method of soil survey. Rigid grid method is a survey method in which points of observation were fixed at the intersection of perpendicularly running transects. The transverse were spaced 50 m apart. After the mapping exercise soils with similar characteristics were grouped together as the same mapping unit. Four soil types were identified Soil profile pits were located and dug to represent each of the soils identified on the field. Altogether fifteen soil samples were collected from the four profile pits. For the soil profile samples collected, notes were taken on soil colour, texture, structure, consistence, stoniness, mottles, cutans and concretions.

Laboratory analysis: Samples were prepared and routinely analyzed following the guidelines of IITA [11].

Soil classification: The soil identified were classified according to Smyth and Montgomery [12] at the series level, Soil Taxonomy (Soil Suvey Sytaff) [13] and (FAO/UNESCO) [14].

RESULTS AND DISCUSSION

Morphological and physical characteristics: The important morphological characteristics of the soil are given in Table 1. The pedon A has a yellowish red (5YR 5/6). A horizon coming down to redish yellow (5YR 6/8) sub soil. Pedon B has a darkbrown (10YR 4/3). A horizon coming down to a yellow (10YR 7/8) subsoil. Pedon C has a grayish brown (10YR 5/2) topsoil coming down to light reddish brown (2.5Y 6/3) subsoil, while pedon D has a very dark grayish brown (10YR 3/2) topsoil coming down to yellowish red (5YR 5/8) subsoil. From all the descriptions above subsoil matrix colour in the solum is redish, brownish or yellowish brown with occasional strong brown yellowish brown or yellow or red mottles. Reddening of upland subsoils is considered due to mobilization and subsequent immobilization of iron during redox-cycles in the soils [15] causing dispersion with progressive oxidation of iron [7] postulated a biogenetic process for the colouration, due to mechanical mixing by plant roots and soil Fauna (termites mainly). The bright subsoil colour in some of the profiles may be an indication of good internal drainage. Nearly all the soil profiles are highly mottled due to lack of mechanical mixing by plant roots and soil Fauna.

Surface soils are generally coarse loamy sand/sandy loam, grading into loamy sand/sandy loam/sandy clay loam (Table 2). Percentage sand content is high virtually in all the profiles indicating the sandy nature of the parent material. For profile B, there is percentage clay increase probably justifying the recognition of argillic horizon [16] observed that marked weathering and argillation of bedrock to saprolite had taken place in the Basement Complex of SW-Nigeria. Sesquioxidic concencretions [17], ferruginous skeleton [7], Iron nodules and concencretions [5], petroplinthite [18] and quartz materials are dominant in the gravelly horizons of the solum. These are indicative of different climatic or bioclimatic regimes as suggested by Amusan and Ashaye [4]. Dikerman and Medema [19] noted that the presence of iron stone gravels in-situ in residual upland soils suggests that there had at one time been conditions favourable for the segregations of iron possibly in the form of plintholes. This process requires an adequate supply of iron, alternating wet and dry seasons, a relatively flat land surface with seasonally wet soils such as occur in the study area. Thomas and Thorp [20] remarked that cyclic change of climate occurred during soil formation in West Africa.

Physico-chemical properties of the soils: The Ap horizon of all the pedons have high sand content (>75%) with low clay content except for pedon B. An outstanding feature of these soils irrespective of their location on the topography is the moderate to high silt content (11-17%) at the surface. This characteristic distinguishes these soils from most other sandy soils of southwestern Nigeria, characterized by low silt (<10-15%) content-21, 2002, Shittu and Fasina 2004. This high silt content reflects the fact that most of the soils were formed from coarse colluvial materials (Hillwash). This tends to suggest that erosion is a marked pedogenic process in this area.

For pedons B and C, the particle size of the subsoil horizons of the soils suggest that the B-horizons are influenced more by the rate of soil weathering and eluviation-illuviation process. The high clay content in the deeper horizons of some of the soils (profiles B and C) coupled with some morphological properties (Table 1)

Table 1: Field morphological description of pedon studies

Depth (cm)	Colour (Hne) (Mnist)	Mottles +++	Texture + + + +	Structure * *	Consistence Dry + Wet	Cutans k k	Drainage Class k k k	Concretions + + + +	Roots ++
Pedon A									
Apomu									
0-20	5yr 5/6	-	Between to Sl	Cr, Sab	d, L	-	IV	Pm Fe-Mn	M
20-60	5yr 6/8	M, yellow, red	LS	Ls	C r	F	IV	Pm Fe-Mn	F
60-125	5yr 6/8	M, yellow, red	LS	Sab, ab	h	F	IV	Pm Fe-Mn	F
Pedon B									
Iregun									
0-30	10yr 4/3	F, red	Sl	Cr,gr sab,	Sh	-	I	-	M
30-60	5yr 6/8	F, red	SL	Ab	h	F	I	Pf, Fe-Mn	F
90-130	10yr 7/8	F, yellow, red	SCL	sab, ab	h	F	I	Pf, Fe-Mn	-
Pedon C									
Balogun									
0-12	10yr 5/2	M, yellow; f, red	Sl	Sab, ab	Fr	-	I	-	M
12-28	10yr 7/4	F, yellow	SL	Sab, b	M	F	I		F
28-33	2.5y 6/4	F	SL	Sab,b	M	F	I	Pf, Fe-Mn	-
33-107	2.54 6/13	M, yellow	SL	ab	M	F	I	Pm, Fe-Mn	-
107-173	2.54 6/3	M, yellow	SC:	Sab, ab	M	M	I	-	-
Pedon D									
Apomu									
0-28	10yr 3/2	F, red	LS	CR	FR	-	IV	-	M
28-94	7.5YR 5/6	F, red	SL	Sab, ab	h	F	IV	Pm Fe-Mn	F
94-133	5yrs 6/8	M, yellow, red	SL	Sab, ab	h	M	IV	Pm	-
133-180	5 yr 5/8	M, yellow,	SL	Sab, ab	h	M	IV	Pm	-

Table 2: Physical and chemical properties of pedons studied

									Exchangeable cations (Cmol kg^{-1})				Exch			Organic
Sample	Gravel (%)	Sand (%)	Silt (%)	%/Clay	Texture	pH 1:2 H 20	Total N (%)	Avail P (ppm)	Ca	Mg	Na	K	acidity (Cmol kg^{-1})	ECEC Soil	B.S (%)	carbon (%)
Depth (cm)																
A Pedon A Apomu																
0-20	48.13	75	15	10	Sl	5.36	0.32	1.68	3.2	0.36	0.28	0.18	1.2	85.98	85.98	0.64
20-60	55.77	81	9	10	LS	5.70	0.12	3.58	2.6	4.0	0.39	0.17	1.6	8.76	81.74	0.24
60-125	80.57	77	17	6	LS	5.59	0.07	3.47	3.2	5.2	0.35	0.13	0.4	9.28	95.69	0.14
B Pedon B																
Iregun																
0-30	15.83	75	11	14	Sl	5.89	0.02	4.26	3.6	1.1	0.29	0.07	1.6	6.66	75.98	0.04
30-90	38.78	69	15	16	SL	5.06	0.06	6.50	2.2	5.3	0.30	0.09	1.0	8.89	88.75	0.12
90-130	45.79	59	15	26	SCL	5.30	0.01	0.37	3.4	4.5	0.35	0.16	1.6	10.01	840.2	0.02
Pedon C																
Balogun																
0-12	6.85	75	17	8	SL	4.90	0.14	2.91	1.5	3.6	0.31	0.10	1.6	7.11	77.50	0.28
12-28	26.71	75	15	10	SL	5.38	0.16	2.24	2.0	4.2	0.35	0.06	0.4	7.01	94.29	0.32
28-33	48.57	69	15	16	SL	6.07	5.60	1.8	3.8	0.50	050	0.07	0.2	6.37	96.86	0.14
33-107	39.41	55	27	18	SL	8.14	0.01	4.70	2.3	9.67	1.20	0.06	0.2	13.36	98.50	0.02
107-173	57.42	61	19	20	SCL	8.12	0.06	1.68	3.2	5.7	0.96	0.07	0.6	10.53	94.30	0.12
Pedon D																
Apomu																
0-28	15.91	81	13	6	LS	5.88	0.27	4.36	2.9	3.0	0.31	0.11	2.0	8.32	75.96	0.54
28-94	49.40	77	13	10	SL	6.16	0.01	2.13	2.5	3.0	0.30	0.15	3.2	9.15	62.02	0.02
94-133	74.76	65	17	18	SL	6.11	0.10	3.70	3.5	3.3	0.41	0.23	2.8	10.24	72.66	0.18
133-180	58.39	63	19	18	SL	5.79	0.03	1.57	3.8	1.8	0..37	0.28	2.0	8.25	75.76	0.06

and 2) formed the basis for the recognition of argillic horizons in some of the soils.

Another property that is likely to affect crop production in these soils is the high percentage level of concretions and gravels. The depth of gravel content varied widely for all the profiles with values ranging from 6.85%-48.13% on the surface. Percentage concretions/gravels increased with depth (Table 2) in all the profiles. Many to common iron/manganese (Fe/Mn) concretions occur in most of the profiles (Table 1 and 2) right from the soil surface. This perhaps is an indication of alternating wet and dry cycles. This might in fact infer that pliinthization may be another pedogenic process responsible for the soil development of this area. The gravel content observed is a common feature of those soils formed in the upland portion of landscapes derived from granitic metamorphic rocks of central southwestern Nigeria [12, 17]. The high gravel/concretions may constitute a serious problem to crop production within the study area.

The soils are strongly acidic (4.90-profile C) to moderately acidic (5.89-profile B) at the surface. For profile C, the soil was moderately alkaline (8.12-8.14) in the subsoil. Factors suggested to be responsible for the acidic solum may include heavy rainfall, the acidic nature of the parent rock and the acidic precipitation around the study area. Annual rainfall around the study area is about 1367 mm [22] and most of this fall within five to seven months in the year. This distribution of rainfall is considered adequate for leaching and colloid translocation. Precipitation within the study area is acidic [22] and is believed to enhance soil acidity.

The organic matter content of the soil is generally low even less than 2% with the surface soil horizons consistently containing the highest amount Soil organic matter decreases with depth down the soil profile in virtually all the profiles expect profile C. The low organic matter obtained may be partly due to the effect of high temperature and relative humidity which favour rapid miniralization of organic matter. The low soil organic matter on the surface might also not be unconnected with the degradative effect of cultivation and other land use and management activities. The low organic matter content recorded on the average for most of the soils cannot sustain crop production program. Therefore, the organic matter content have to be substantially increase through effective crop residue management.

The nitrogen contents in the soils are on the average low with higher values concentrating in the top soils (0.32%-PA and 0.27-PD). The values of nitrogen decreased with increasing depth in most of the profiles. These nitrogen values obtained can be considered low to medium when compared to the critical value of plant nutrient for Nitrogen (0.20 kgG^{1}) for most tropical crops. These values were also in agreement with values recorded for Nigerian soils [21, 23, 24]. These values are generally low and the inadequate needs to be taken into consideration in managing these soils.

The effective action exchange capacity (ECEC meqlioosil) values of the soils are low. The low ECEC values indicate that the soils have low potential for retaining plant nutrients, hence the necessity for adequate soil management. These values are low when compared with values reported for Nigerian soils [21, 23, 24]. These low ECEC values, coupled with low pH and organic matter, are indications of low, inherent soil fertility status which underscores the need for improved soil management techniques, in addition to the traditional methods of bush fallowing, if crop production is to be sustained. Considering the critical values of exchangeable Mg (0.2 meqi/100g. soil) and 0.15 meq/100g soil for most crops, these two exchangeable bases appear adequate. The high levels of Exc Ca and Mg in these soils tends to suggest that the parent material from which the soils are formed contains these nutrients.

Available P is generally low and considered inadequate for crop production when compared with critical values recommended for most tropical crops (8-15 ppm) [25].

Soil genesis: A critical study of these soils reveal that hydrolytic weathering plays a significant role. High rainfall, high soil temperature, acid precipitaiton and goechemical nature of the parent rock all seems to encourage hydrolytic weathering since precipitation is higher than 0.8PET [22] in this region, leaching of basic cation is encouraged. High temperature encourages photolysis of water. Ojanuga [26] remarked that warm soil temperature causes marked dissolution of soil water, ending to a build up of hydrogen ions. Siever [27] reported that high soil temperature and extreme leaching favour rapid desilication and accumulation of iron, inmobilized in ferric oxide forms under oxidixing conditions. Most of the pedogenetic processes that do occur within the study area are being favoured by humid conditions, free internal drainage, geomorphic stability over prolonged period of time and strong weathering intensity [28]. In the Basement complex region of SW-Nigeria, the climate is humid as precipitation is greater than ET-for-greater part of the year with continually high

temperature. It therefore appears that both the climatic, geologic and physical setting of the region predispose the parent rock to Ferrallitization [29] which is the basis of ferrallitic pedogenesis. Tessens and Shamshuddin [29] noted that ferralitic weathering is the dominant soil forming process in the humid tropical regions. Lessivation, braunification, induration, floral and faunal pedoturbation, erosion, mobilization and subsequent immobilization of iron during redox cycles in soils and cyclic change of climate were considered as other dominant pedogenetic processes in the soils.

Soil classification: The classification of the soil was done according to the USDA Soil Taxonomy [30] and series system of [12]. Pedon A is distinguished by the presence of granular to crumby structure, texture ranger from sandyloam on top coming down to loamy sand. No major diagnostic horizon was noticed. These soils were formed on coarse colluvial texture materials with clay content less than 15% throughout the soil profile. The soils in this group have been classified at first level as Entisol. The presence of a texture of loamy fine sand or coarse in all layers within the particle size control section necessitates the classification of this pedon into suborder "psamment" and at the second level (subgroup) as Typic Udipsament and as Ferralic Arenisol with evidence of yellowish mottles at 20-60 cm. There is also presence of iron and manganese concretion from the topsoil to the lowest region (Table 1). They are classified as Apomu series at series level. This is because they displayed drainage mottling in pale yellowish and rusty orange brown colours. They are sandy in nature to a depth of more than 100cm. They are the concretionary variation of Apomu which are exceptional [12] in that they contain few to fragment large, knobby concretions, some of which was formed above a depth of 18-20 inches in this profile. They are large fragments of broken hardpan which from their rounded surfaces appear to have undergone partial resolution.

On the basis of the criteria of the USDA Soil Taxonomy [13], Peddon B have argillic horizon in the texture range with low base saturation and low apparent ECEC (<+99% by sum of bases method as documented by [30]<12 cmol(+) kg^{-1}clay) within the B horizons and the fufilment of the requirement of clay content increase with depth. Peddon B have been classified at first level as Ultisol because of their low base saturation<99% by sum of bases [30]. The prevalent Udic moisture regime in the ultisol necessittaes their classification into the suborders 'udult'. There is presence of iron and manganese concretions within 150 cm of the surface which made pedon B to be classified at second level as Typic Plinthudult and as Dystic Pilmthosol and Iregun Series.

Pedon C has been classified as an Inceptisol-young soil. There is slight increase in clay with depth though not appreciable. They have low base saturation (<99% by sum of bases method-soil survey staff 1990) and low ECEC (<12 cmol kg^{-1}). There is also precence of Fe-Mn concretions at 28-107cm with evidence of yellowish red mottles throughout the soil profile: The moisture regime is Udic. The soil has been place in suboder Udept and great group Dystrudept and subgroup Typic Dystrudept. It was classify as Ferralic cambisol [14] and as Balogun Series [12].

The last group of soils (Pedon D) has low base saturation (<99%) by sum of bases method and Low ECEC (<12cmol kg^{-1}). The moisture regime is Udic. The soil is highly weathered and leached as evidence by the soil colour and values of the Exchangeable soil bases in subsoil (5YR 5/8)-yellowish red). (Table 1). At first level the soil was classified as an oxisol and suborder Udox and great group Hapludox. At second level it was classified as Plinthic hapludox and Plinthic Ferralsol [14] and as Apomu Series [12].

Sustainable management of the soils: Most soil management practices are largely limited to the ploughed layer. For instance, it was found that 70% of crop yield variability were due to soil properties that occur in the ploughed layer [31].

The soil acidity warrants lime application especially in the subsurface layers which support the roots of most of the arable and tree crops. The organic matter which is low has to be substantially increased through effective crop residue management, increased use of leguminous plants as well as judicious use of organic fertilizers.

Appropriate use of chemical fertilizers will ameliotrate exchangeable bases limitations, but the use of ammonium fertilizers should be strictly guided against to prevent erosion and leaching. Post harvest incorporation of plant residue into the soil instead of the usual burning of crop residue to stimulate the emergence of new flushes for grazing will stabilize the soil aggregates. Minimum tillage is recommended because of the concretonary nature of the soils. The soil with hardpan concretions must always be under cover to prevent serious erosion.

In conclusion the framework for evaluating sustainable Land Management (FESLM) as proposed by Smyth and Dumanski [32] can be adopted in the sustainable management of these soils.

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Semi-Empirical Models Relating Soil Hydraulic Characteristics and Solute Breakthrough Curve

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Abstract: Since the convection of a fluid in different pores is described by the pore velocity distribution and consequently the pore size distribution, it is possible to connect the BTC to the pore size distribution or the SMC. In this study we presented an approach relating the CDE and Kosugi's SMC model by the simple and easily measured parameters. The BTC and SMC were measured simultaneously in core scale soils under laboratory condition. These two models were connected together with 2 arbitrary constants and 2 empirical coefficients. The obtained SMC and BTC models based on the empirical coefficient were evaluated in wide range of soils. The correlation coefficient between measured and predicted effective saturation and relative effluent concentration ware found 0.9215 and 0.9299 respectively. The RMSEs of predicted and experimental data ranged from a low of 0.031 to a high of 0.177, the averaged being 0.0802 for SMC and from a 0.031 to 0.243 and the averaged being 0.0899 for BTC. As the RMSR for BTC and SMC didn't show any correlation with soil physical and hydrodynamic properties, we resulted that these models have enough flexibility in the wide range of soil properties.

Key words: Solute break through curve . water retention characteristic curve . modeling

INTRODUCTION

Because the Soil Moisture Characteristics curve (SMC) is difficult and expensive to measure, many models have been developed to estimate SMC from other soil properties including soil particle size distribution, particle density, ρ_b and soil morphology [1, 2]. Despite the large number of such studies there is no study estimate the SMC from Solute Breakthrough Curve (BTC). On the other hand, to predict the solute transport events as well as SMC, many investigators have shown that the soil physical properties such as the average pore water velocity [3, 4], the pore size distribution [5, 6, 7] can determine the transport phenomena. Several attempts have been made to relate the soil physical properties with the BTC models parameters. Computer simulations by Vogel, (2000) suggested that solute dispersion is more sensitive to the water-retention curve than the pore-size distribution. Dispersivity is a key parameter in solute transport models and has partially been related to the slope of the SMC [7, 8] and to multiple slope of SMC and the air entry value [9].

Other researcher has proposed an experimental relation between dispersivity and variance of pore water velocity distribution based on the HCC [10]. Databases of soil hydraulic properties are widely available [11, 12, 1]. Similarly if solute breakthrough curves and soil hydraulic properties were measured simultaneously on samples from a wide range of soil types, pedotransfer functions [13] could be developed to predict dispersivity from information stored in existing databases. Goncalves *et al*. (2001) have developed PTFs to predict transport parameters from basic soil properties and SMC parameters using multiple linear regression and neural network analysis. PTFs are experimental regression equations that are used to predict difficult-to-obtain parameters from more easily measured soil properties.

Wang *et al*. (2002) successfully have used Brooks and Corey's water retention model to derive a simple model predicting the BTC in disturbed sand columns under saturated conditions.

Since the convection of a fluid in different pores is described by the pore velocity distribution and consequently the pore size distribution, it is possible to connect the BTC to the pore size distribution or the SMC. At the core scale, solute transport is often described by means of the Convection Dispersion Equation (CDE). The hydrodynamic dispersivity in this equation reflects the microscopic variability of solute transport on top of the mean convective flow.

In order to describe the SMC, many models have been developed [14, 15, 16, 17]. The Kosugi's model

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is based on lognormal pore size distribution of soil and it has two physical meaningful parameters. Since the CDE and this model have been used frequently and have similar mathematical form, so relating between these two models and their parameters will be more valuable and useful than complete empirical pedotransfer function which relating soil physical properties and CDE or SMC parameters. Also in many cases at core scale, the measuring of BTC is easier than the measuring of SMC, consequently any reliable relation between the BTC and SMC can result in easily predicting of SMC too. The purpose of the current research are (i) to present an approach that can relate the CDE and Kosugi's SMC model by the simple and easily measured parameters and (ii) to obtain and evaluate the empirical coefficient of these models in wide range of soils under laboratory conditions.

Model development: We assume that at any time, the C/C_0 in outlet point is a function of relative portion of the pores discharching the solute to the all pores contributing in water transport. According to their sizes, coarser pores carry the applied pulse concentration to outlet point sooner than fine pores and with increasing time the solute of finer pores reaches to outlet point too. i.e. At any depth the portion of solute carrying pores increases with time where the arriving time of the each pore solution depends on its size, consequently we assume the C/C_0 at outlet point is function of pore size distribution or SMC. This may be expressed as:

$$C/C_0(t) = f(Se) \tag{1}$$

One dimensional transport of solute through homogeneous soil during steady water flow is traditionally described by CDE:

$$R\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - V\frac{\partial C}{\partial x} \tag{2}$$

Where C(x, t) is solute concentration in soil solution, V is average pore water velocity, D is convection dispersion coefficient, t is time, x is space coordinate and R is retardation factor. Initial and boundary condition for semi-infinite column experiment are

$$C(0,t) = C_0 \quad C(8,t) = 0 \quad C(x,0) = 0 \tag{3}$$

Where C_0 represents a constant concentration of solute supplied at column inlet. The solution of Eq. (2) under condition of Eq. (3) is well known [18]:

$$\frac{C}{C0} = \frac{1}{2}\text{erfc}\left[\frac{R - Pv}{(4RPv/Pe)^{1/2}}\right] + \frac{1}{2}\exp(Pe)\text{erfc}\left[\frac{R + Pv}{(4RPv/Pe)^{1/2}}\right] \tag{4}$$

Where erfc is complementary error function, 'Pv' is number of pore volume equal toVt/L, L is soil column length, Pe is column Peclet number that equal toVL/D and R is given by:

$$R = 1 + \rho_b.K_d/\theta \tag{5}$$

ρ_b is soil bulk density and. K_d is linear adsorption coefficient.

As the percentage contribution of second term in right hand of Eq. (3) to total relative concentration is negligible [19] so we can consider only the first term as BTC model.

The soil moisture characteristics curve model developed by Kosugi, (1996) is:

$$Se = \frac{1}{2}\text{erfc}\left\{\frac{\ln(\psi) - \ln(\psi_0) - \sigma^2}{2^{1/2}\sigma}\right\} \tag{6}$$

Where ψ_0 is pressure at inflection point of pore size distribution curve, σ^2 is variance of pore size in lognormal distribution,

$$Se = \frac{\theta - \theta_r}{\theta_s - \theta_r}$$

Which θ_s and θ_r are saturated and residual water content respectively.

Substituting the Eq. (4) and Eq. (6) in Eq. (1) yields;

$$\begin{aligned}\frac{C}{C0} &= \frac{1}{2}\text{erfc}\left[\frac{R - Pv}{(4RPv/Pe)^{1/2}}\right] \\ &= f\left(\frac{1}{2}\text{erfc}\left[\frac{\ln(\psi) - \ln(\psi_0) - \sigma^2}{2^{1/2}\sigma}\right]\right)\end{aligned} \tag{7}$$

Consequently the function between SMC and BTC expressed in Eq. (7) can be represented as:

$$\left[\frac{R - Pv}{(4RPv/Pe)^{1/2}}\right] = F\left(\frac{\ln(\psi) - \ln(\psi_0) - \sigma^2}{2^{1/2}\sigma}\right) \tag{8}$$

Figure 1 may show the C/C_0 as function of second and third term of Eq. (7) in provided that f(x) = x. Figure 1 shows the shape similarity between the SMC and BTC of a nonreactive solute in a soil of our experiments. Figure 1 shows that in addition of soil

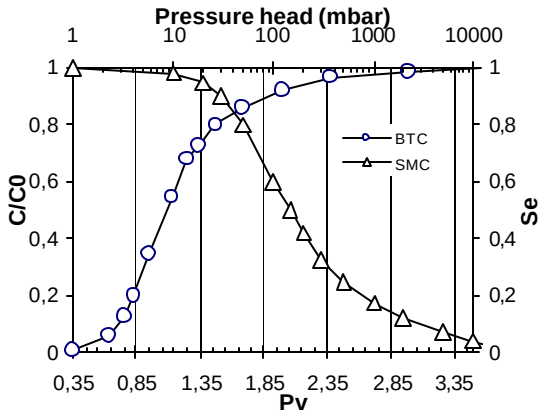

Fig. 1: Shape similarity between SMC and BTC in a Loam soil (marked with ¶ in Table 1). C/C_0 is drawn as function of 'Pv' and ψ as described in Eq. (6) provided that the 'a 'and 'b' in Eq. (9) are defined.Note to logarithmic scale of ψ

hydraulic properties, the type of function (f) depends on dimension and scale of 'Pv' and 'ψ'. So it is necessary to unify and agree between the 'Pv' and 'ψ'' for finding the "f "or "F" .i.e. because the Eq. (8) has two variable parameters in both side, fixing one parameter against another is needed to understand the relationship. This agreement between 'Pv' and 'ψ' can be defined with a exclusive equation such as:

$$Pv = a\psi^{b} \qquad (9)$$

The 'a' and 'b' in Eq. (9) are arbitrary constant parameters and must be chosen as the Eq. (9) gives the reliable answers in 'Pv' and 'ψ' given range and leads to simplify the function "F" as possible.

The function (F) may become linear in valid value of a and b. Consequently the Eq. (8) can be represented:

$$\left[\frac{R-Pv}{(4RPv/Pe)^{1/2}}\right] = p\left(\frac{\ln(\psi)-\ln(\psi_0)-\sigma^2}{2^{1/2}\sigma}\right)+q \qquad (10)$$

Where, 'p' and 'q' are constant coefficient and depend on soil physical and hydrodynamic properties. For simplification it may express by:

$$Y = pX + q \qquad (11)$$

Finding two different point of this line [X1, 0] and [0, Y1] can give the value of 'p' and 'q'.
If X = 0 in Eq. (11) then

$$\frac{R-Pv}{(4RPv/Pe)^{1/2}} = q \qquad (12\text{-}1)$$

or

$$\ln(\psi) = \ln(\psi_0) + \sigma^2 \qquad (12\text{-}2)$$

And where Y = 0 then Pv = R or

$$\frac{\frac{\ln(R)-\ln(a)}{b}-\ln(\psi_0)-\sigma^2}{2^{1/2}.\sigma} = -\frac{q}{p} \qquad (13)$$

If SMC data be available, 'p' may be estimated with soil physical properties. Since R can be determined from batch studies as described in Eq. (5), 'q' can be calculated with Eq. (13). Consequently predicting of BTC will be possible with using Eq. (10).

On the other part, the p may be estimated when the BTC data is available. Eq. (10) can be represented as:

$$\frac{R-Pv}{(4RPv/Pe)^{1/2}} = \frac{p}{2^{1/2}\sigma}\ln(\psi) - p\left(\frac{\ln(\psi_0)+\sigma^2}{2^{1/2}\sigma}\right)+q \qquad (14)$$

Considering the defined relationship between 'ψ' and 'Pv' the measured value of $\frac{R-Pv}{(4RPv/Pe)^{1/2}}$ and ln(ψ) may be resolved in Cartesian coordinate with Y and X axis respectively, so for any reliable amount of R, Pe and 'ψ', this equation gives a line with known slope S and intercept I as:

$$I = q - p\left(\frac{\ln(\psi_0)+\sigma^2}{2^{1/2}\sigma}\right) \qquad (15)$$

$$S = \frac{p}{\sqrt{2}.\sigma} \qquad (16)$$

Since the slope can be derive with above resolving and value of 'p' are estimated with BTC, the value of σ can be calculated with Eq. (16).
Combining the Eq. (12-1), Eq. (12-2) and Eq. (9) yields:

$$q = \frac{R - a(\psi_0.e^{\sigma^2})^{b}}{\left(\frac{4R.a(\psi_0 e^{\sigma^2})}{Pe}\right)^{1/2}} \qquad (17)$$

Substituting the Eq. (17) in Eq. (15) gives:

$$I-\frac{R-a(\psi_0.e^{\sigma^2})^b}{\left(\frac{4R.a(\psi_0 e^{\sigma^2})}{Pe}\right)^{1/2}}+p\left(\frac{\ln(\psi_0)+\sigma^2}{2^{1/2}\sigma}\right)=0 \qquad (18)$$

Only the ψ_0 is unknown parameter in Eq. (18) and can be calculated easily by a simple numerical method.

METHODS AND MATERIALS

28 columns were filled from the 0 to 18-cm depth (Ap) of different soils. Sampling locations were selected on the basis of textural class to give a wide range of soil hydraulic properties. Soil samples were all obtained from the north and centre of Iran. The 5.0 -cm radius by 30-cm long PVC soil columns were filled with dried and sieved by 10 mesh sieve, then soils were saturated from below. Miscible-displacement experiments was determined for each column with continues applying the 0.1 M $CaCl_2$. SMC were measured using the standard procedure [20], with unifying the bulk density in each column and revealed core sample. SMC and BTC parameters were obtained by MATLAB 7.1 software.

RESULTS AND DISCUSSION

In order to obtain any relationship between BTC and SMC, it is necessary to define or find reveal value of 'a' and 'b'. Figure 2 shows the type of relationship (function "F") between two side of Eq. (8) in different values of 'a' and 'b'. As the shown in Fig. 2b, with decreasing the 'b' from 1 to 0.25, the function F in Eq. (7) becomes linear but in low amount of 'b' (<0.25) the answers of Eq. (8) and Eq. (9) in any amount of 'a' becomes unreliable. So the 0.25 may chosen as the correct value of 'b'. Fig. 2a represents the mentioned relationship type as function of 'a' with chosen value of 'b' (0.25). In high and low value of 'a', the function "F" is nonlinear and it becomes linear when a shifts to 2. Then we consider 'a' and 'b' as 2 and 0.25 respectively where pressure head expressed as mbar. These constants value gives a linear and reliable relationship between BTC and SMC parameters as described in Eq. (10) in any valid amount of soil physical and hydraulic parameters with R^2>0.99. (Detail is not shown).

As shown in Eq. (13) through (16), 'p' is a key parameter and it may be estimated by correlating with soil physical properties. As any strong and significant relation was not observed between 'p' and single physical properties, the combination of hyadraulic parameters were used. The following relationship were obtained between the parameter 'p' and the complex of soil properties and hydraulic parameters (Fig. 3):

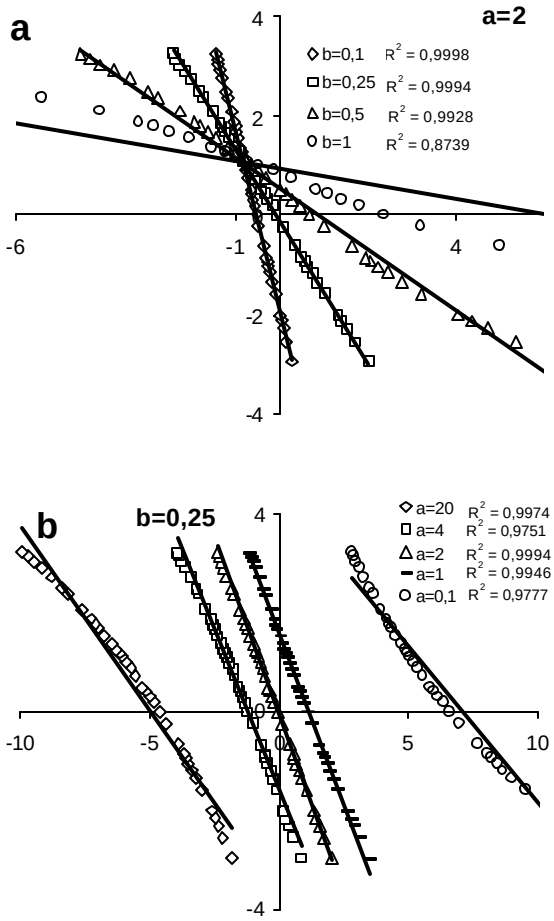

Fig. 2: Function type ("F") between the two side of Eq. (7) in different value of 'a' and 'b' in soil No 2. (R = 1, Pe = 24.8, $\ln(\psi_{0(cm)})$ = 1.0118, σ = 3.0944 (cm)) the linearity is shown with correlation coefficient derived with fitting line on the data. The X axis is the $\frac{\ln(\psi)-\ln(\psi_0)-\sigma^2}{2^{1/2}\sigma}$ and the Y axis is $\frac{R-Pv}{\sqrt{\frac{4PvR}{Pe}}}$

$$\ln(-p)=0.3362\frac{R\sigma^2}{K_s/(\theta s-\theta r)}-0.4666 \quad (r^2=0.8545^{**}) \qquad (19)$$

Which the defined unite of σ and K_s are cm and cm h^{-1} respectively.

The parameter 'p' was found strongly related to Peclet number 'Pv' (Fig. 4) and resulting relationship based on our experimental data is:

$$\ln(-p)=0.508\ln(Pe)-1.0543 \quad (r^2=0.9057^{**}) \qquad (20)$$

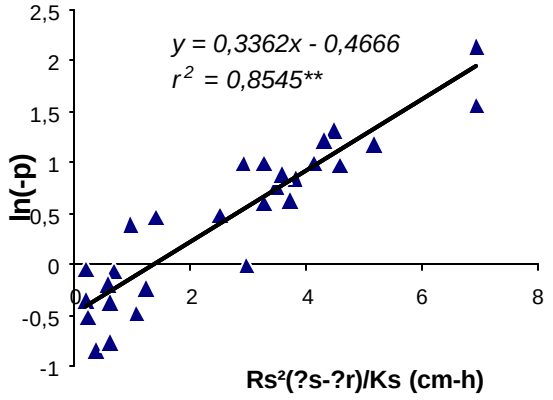

Fig. 3: The relationship between a defined soil physical parameter $\frac{R\sigma^2}{K_s/(\theta s-\theta r)}$ and the parameter 'p'

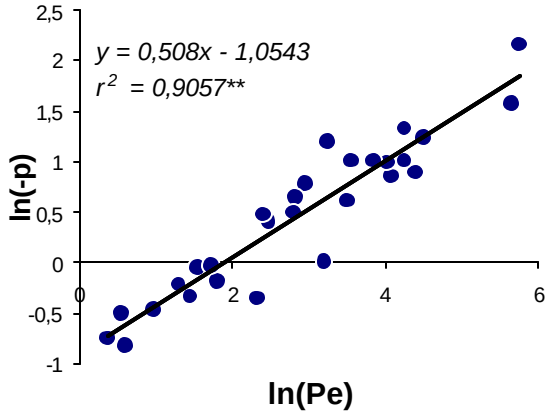

Fig. 4: The relationship between the parameter p and Peclet number Pe

Which can be used in simulating of SMC. The coefficients of Eq. (19) and Eq. (20) are empirical and may be influenced by regional circumstance.

Comparing the Eq. (19) and Eq. (20) represents a relation between soil physical parameters and Pe.

$$\ln(Pe) = 0.6618\frac{R\sigma^2}{K_s/(\theta s-\theta r)} + 1.1569 \qquad (21)$$

This equation can be used for predicting of Pe with $r^2 = 0.821^{**}$.

Evaluation of models: Simulated and observed BTC for 4 soils are shown in Fig. (5a, b, c, d). As shown the model has simulated well both the SMC and BTC with each other.

The empirically estimated 'p' was used in Eq. (10) to independently calculate SMC and BTC of 28 soils.

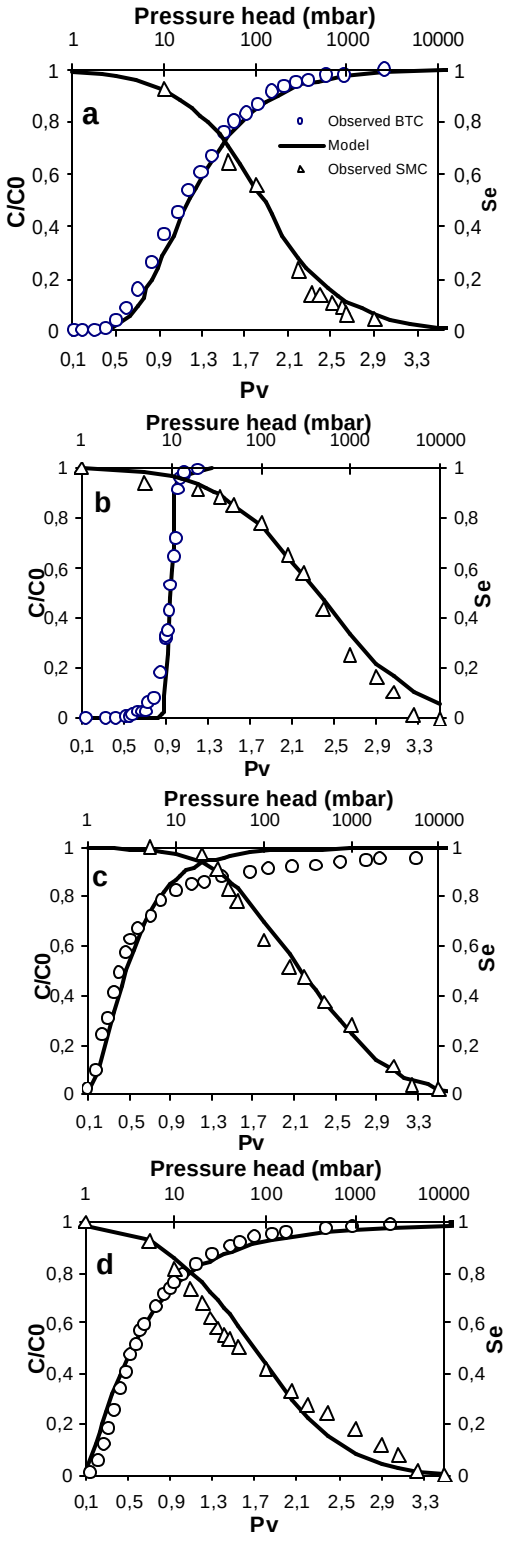

Fig. 5: Observed and simulaed BTC and SMC in 4 different soil. a, b, c and d represent sand, loam, clay loam and silty clay soils respectively. These soils are marked in Table 1 with †

Table 1: Soil type and Parameters of Eq. (10) that relate the breakthrough curve to soil moisture characteristics curve with their respective Root Mean Square Residuals (RMSR of measured and predicted value of C/C_0 and Se respectively)

Soil	Texture	p	q	RMSR for predicting SMC	RMSR for predicting BTC
Quartzipssament	Sand	-1.067	0.3578	0.117	0.098
Quartzipssament	Sand	-0.991	-0.167	0.097	0.073
Quartzipssament	Sand	-1.444	-0.079	0.081	0.049
Quartzipssament	Sand †	-0.426	-0.896	0.115	0.0508
Quartzipssament	Sand	-3.372	0.468	0.072	0.127
Torripssament	Loamy sand	-0.37	-0.389	0.142	0.07
Torriorthent	Sandy loam	-0.208	-0.745	0.116	0.117
Torriorthent	Sandy loam	-2.494	-0.97	0.064	0.087
Torriorthent	Sandy loam	-1.965	-0.502	0.055	0.056
Haplocacid	Loam	-1.651	-0.535	0.077	0.09
Haplocacid	Loam	-1.825	-0.518	0.058	0.126
Haplocacid	Loam	-2.727	-0.604	0.031	0.089
Haplocacid	Loam¶	-0.266	-0.594	0.041	0.031
Calcixerept	Loam	-0.465	-0.508	0.052	0.062
Calcixerept	Loam†	-0.115	-0.637	0.041	0.08
Calcixerept	Loam	-0.669	-0.675	0.052	0.243
Calcixerept	Loam	-0.622	-0.596	0.047	0.059
Calcixerept	Loam	-2.075	-1.33	0.04	0.064
Calciargid	Loam	-0.529	-0.441	0.06	0.156
Cacixeroll	Sandy clay loam	-0.368	-0.539	0.045	0.059
Cacixeroll	Silty clay loam	-1.017	-0.456	0.094	0.068
Argixeroll	Silty clay loam†	-1.238	-0.523	0.07	0.046
Argixeroll	Clay loam	-2.238	-1.244	0.047	0.062
Argiudoll	Clay loam †	-1.1758	-1.005	0.039	0.149
Calciargid	Clay loam	-2.168	-2.269	0.177	0.161
Haploxeralf	Silty clay	-1.039	-0.947	0.173	0.057
Argiudoll	Clay	-3.44	0.037	0.08	0.088
Argiudoll	Clay	-2.727	-0.595	0.081	0.065

†BTC and SMC of these soils are presented in Fig. 5a, b, c, d, ¶ BTC and SMC of this soil is presented in Fig. 1

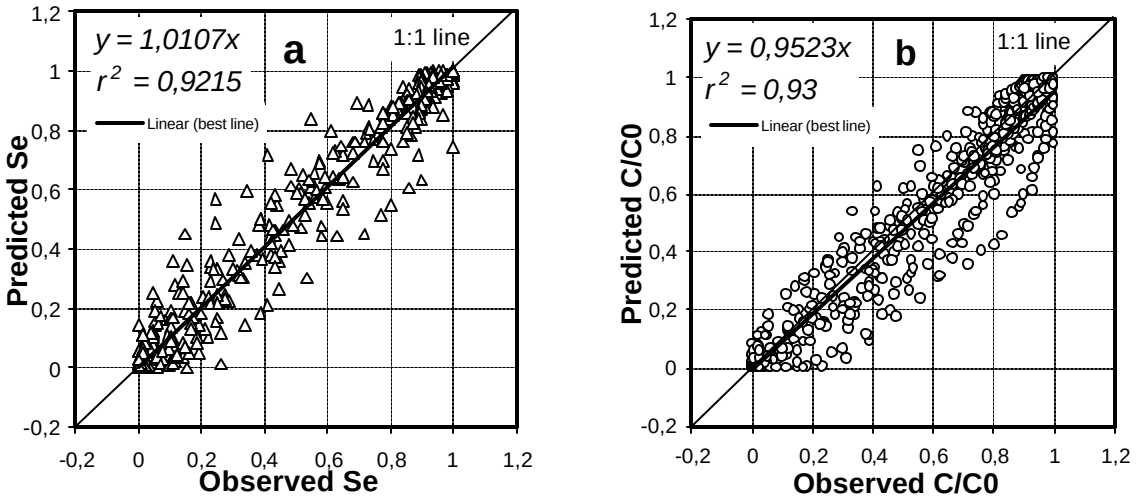

Fig. 6: Comparision of predicted and observed C/C_0 and Se. Test results for 28 soil are pooled

These soils are identified in Table 1. Examples of predicted and experimental data are presented in Fig. 5a (sand), 5b (loam), 5c, (silt loam) and 5d (clay). Overall, the shapes of the predicted SMC and BTC were similar to those of the measured data in wide range of soil physical properties.

Figure 6 shows a comparison of experimental vs. predicted SMC (Fig. 6a) and BTC Fig. (6b) values for all soils on a 1:1 scale. The regression line between the experimental and predicted values closely matches the 1:1 line with an r^2 of 0.9215 and 0.9299 for SMC and BTC respectively. The Root Mean Square Residuals (RMSR) between predicted and measured C/C_0 and Se values represent respective level of agreement between the model and experimental data. The RMSRs of the predicted and experimental Se data ranged from 0.031 to 0.177 (average 0.0802) and for C/C_0 ranged from 0.031 to 0.243 (average 0.0899), Table 1. As the RMSR for neither BTC and nor SMC show any correlation with soil physical and hydrodynamic properties, we resulted that the presented models have enough flexibility in the wide range of soil properties.

The scatter in the data in Fig. 6a and 6b is not surprising in view of the many sources of variation in the experimental as well as input data. These variations may be attributed to variations in determining the coefficients 'p' and 'q' empirically which can be resulted from (i) neglecting the second term of CDE model (ii) lack of fitting in describing of SMC with Kosugi model (iii) differences in mineralogy, microaggregation and organic matter content. We believe that the predictions of the BTC and SMC from each other by a semiemperical model are quite reasonable. Predictions of the model may be further improved if the empirical coefficients using in calculation of 'p' found in more wide range of soils.

SUMMARY AND CONCLUSIONS

Research reported here describes a method to relate the BTC to SMC using two empirical coefficients. With using two scaling parameters, which relate Pv to pressure head, the BTC based on CDE model is connected to the SMC based on Kosugi model (1996). The model parameters for 28 soils are determined empirically. Our model requires only two parameters of concern: p and q, while finding the first parameter is sufficient for simulation the BTC or SMC. The behavior of these parameters may be further elucidated as the model is subjected to further tests and scrutiny. Predictions of the SMC or BTC from each other for a number of soils, representing a range in texture, Peclet number, saturated hydraulic conductivity and pore size detritions were reasonable. The RMSEs of predicted and experimental data ranged from a low of 0.031 to a high of 0.177, the averaged being 0.0802 for SMC and from a 0.031 to 0.243 and the averaged being 0.0899 for BTC. As the RMSR for BTC and SMC didn't show any correlation with soil physical and hydrodynamic properties, we resulted that these models have enough flexibility in the wide range of soil properties. Lack of fit the Kosugi model as well as adjusted CDE to experimental data and variability in mineralogy, microaggregation and organic matter content attributes and hydrophysical behavior of individual soils are believed to be responsible for uncertainties in the model's predictions. The reported empirical coefficient may be adjusted in future researches with studying on more abundant and undisturbed soils.

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