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Survey of Some Mushrooms in Al-Taif Governorate of Saudi Arabia

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Abstract: Fourteen species of mushrooms belonging to nine genera were collected and identified from seven localities in Al-Taif Governorate of Saudi Arabia. Al-Rouddof region was found to be the richest locality followed by sised and Al-Shafa. Only one species was recorded in Sad Akrama, Al-Mathnah, Wadi Mihrim and Al-Hada localities.

Key words: Mushroom % Al-Taif % Saudi Arabia

INTRODUCTION

Classification of fungi is always suffering from contradictions. This is referred to the lack of complete knowledge about all the fungal organisms. Higher fungi which are related to Basidiomycotina; especially mushrooms, are regarded as spore droppers referring to the method of spore falling from basidia [1].

The survey of wild mushrooms were reported by different researchers [2 - 17].

Little informations were reported about the mushrooms of Saudi Arabia. So the aim of the present investigation was to identify the wild mushroom in different places of Al-Taif Governorate.

MATERIALS AND METHODS

Survey of mushrooms: Different genera of mushrooms were collected from seven localities in Al-Taif Governorate during the period from December 2002 to June 2003. These localities are presented in the map provided in Fig. 1.

Samples were found in damp places of public gardens or on the decayed roots of the dead trees. Samples were photographed and collected from their natural sites and kept for laboratory identification. The collected fruiting bodies were identified according to Breitenbach and Kranzlin [18]; Ellis and Ellis [19]; Klan [20]l; Pacioni [21] and Phillips [1].

RESULTS AND DISCUSSION

The data presented in Table 1 illustrates a list of the identified wild mushrooms, which were collected from seven localities in Al-Taif Governorate of Saudi Arabia. Fourteen species belonging to nine genera were collected and identified.

Description of collected wild mushrooms: Plate I represent all the identified mushrooms:

***(1)- Agaricus arvensis*:** Cap, subglobose becoming flattened, silky, white, turning yellow when touched. Gills crowded grayish turning pinkish and eventually blackish, with white edge. Stipe, club-shaped, white turning yellow. Edible.

(*2)- Agaricus bisporus*: Cap, white then rose brownish when mature, fleshy, globose or hemispherical then convex. Gills rose-white in young, reddish-brownish in mature fungi. Stipe, white sometimes rose in young specimens. Edible.

***(3)- Agaricus augustus*:** Cap, initially subglobose, flat at top, then convex and enventually flattened, reddish-brown on a yellow-cream background. Gills crowded, free, white then grey, pink and eventually chocolate brown. Stipe, cylindrical, enlarged at base, solid then slightly hollow, white turning yellowish with age. Edible.

***(4)- Lepiota procera*:** Cap, cracked, coarsely scaly, brown. Gills whitish. Stipe, hollow when mature, whitish, ochre, with brown stripes and coarse scales along its entire length. Edible

***(5)- Lepiota rhacodes*:** Cap, wide, grey-brown with woolly filaments or overlapping scales. Stipe, white,

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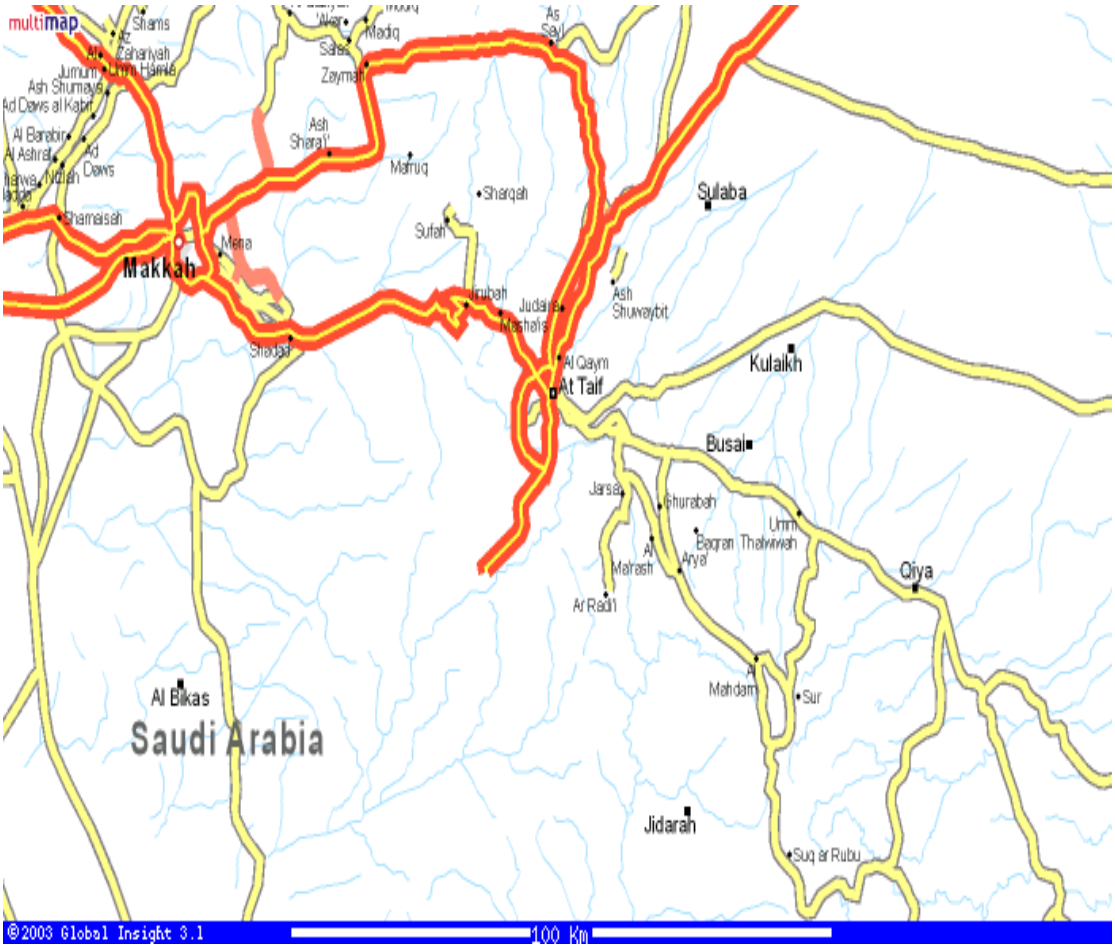


Fig. 1: A map of Saudi Arabia showing the Al-Taif governorate from which the fungi were collected

red-brown when old, smooth, with a bulb white ring. Edible, but may cause gastric upsets in some people.

***(6)- Lepiota cristata*:** Cap, white, with coarse light brown to grey-brown scales. Gills, whitish, free and swollen. Stipe, whitish, brownish at base, smooth, ring white and narrow.

***(7)- Pleurotus ostreatus*:** Cap, often imbricate, superposed violet-black to brownish-grey, fading with age. Gills creamy white. Stipe white smooth, enlarged at top. Edible.

***(8)- Pleurotus cornucopiae*:** Cap, briefly convex and funnel-shaped, whitish or light grey. Gills whitish or cap-coloured, crowded, very decurrent on stipe. Stipe, often ramified, nearly central to fairly eccentric, whitish full, almost completely covered by the extension of gills. Edible.

***(9)- Coprinus comatus*:** Cap, white turning pink at margin then black, cylindrical when young, up to 20 cm in height. Gills white then pink, finally black, free, straight, crowded up to 1 cm long. Stipe, white then dirty white, narrowing towards top, with enlarged rooting base. Edible.

***(10)- Agrocybe cylindracea*:** Cap, pale ochre-brown tending to fade to whitish from margin, convex, silky when dry, often cracked at disc. Gills whitish then greyish brown, fairly crowded. Stipe whitish tinged with pale ochre-brown narrowing towards base. Edible.

(1) Agaricus arvensis

(2) Agaricus bisporus

(3) Agaricus augustus

(4) Lepiota procera

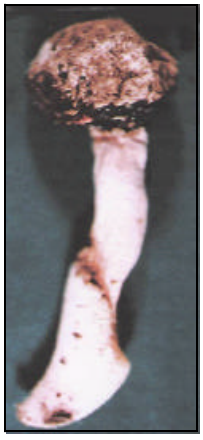
(5) Lepiota rhacodes

(6) Lepiota cristata

(7) Pleurotus ostreatus

(8) Pleurotus cornucopiae

(9) Coprinus comatus



(10) Agrocybe cylindracea

(11) Podaxis pistillaris

(12) Inocybe splendens

(13) Phaeolepiota aurea

(14) Boletus edulis

Table 1: Mushroom collected from Al-Taif Governorate

	El-Roudaf	Secide	El-Shafa	Sad Akrama	El-Masnah	Wady Moharam	El-Hada
1- Agaricus arvensis	-	-	-	-	+	-	-
2- A. bisporus	+++	-	-	-	-	-	-
3- A. augustus	-	-	-	-	-	-	+
4- Lepiota procera	+++	-	-	-	-	-	-
5- L. rhacodes	+++	-	-	-	-	-	-
6- L. cristata	+	-	-	+	-	-	-
7- Pleurotus ostreatus	-	+	-	-	-	-	-
8- P. cornucopiae	+	-	-	-	-	-	-
9- Coprinus comatus	-	+	+	-	-	-	-
10- Agrocybe cylindracea	-	-	-	-	-	+	-
11- Podaxis pistillaris	-	+	-	-	-	-	-
12- Inocybe splendens	-	-	+++	-	-	-	-
13- Phaelepiota aurea	-	+	-	-	-	-	-
14- Boletus edulis	+	-	-	-	-	-	-

- = absent, + = present

***(11)- Podaxis pistillaris*:** Cap, white turning yellow then black, cylindrical. Gills, white, free, straight and crowded. Stipe, white narrowing towards top, with enlarged bulbous base. Edible.

***(12)- Inocybe splendens*:** Cap, first conical with edge raised, then convex with umbo, covered with radial fibrils. Gills whitish then brownish-ochreous, margin paler, adnate, sometimes slightly decurrent. Stipe cylindrical or suddenly enlarged into an almost marginate bulb, pure white finely striate at top. Not edible.

***(13)- Phaeolepiota aurea*:** Cap, golden ochre-yellow, first powdery then valvety and darker, convex. Gills rounded towards stipe, ochreous then rust-coloured, crowded. Stipe cylindrical, slightly enlarged at base, solid, with large ring. Edible, but can cause stomach upset.

***(14)- Boletus edulis*:** Cap, hemispherical, convex then flattened, cuticle smooth, slightly viscous in damp weather, whitish, ochreous, light brown not uniform. Stipe solid, bulging or cylindrical, white or light ochre, covered by a reticulum first white then slightly darker than background. Edible.

Table 1 shows the distribution and frequency of wild mushrooms collected from Al-Taif Governorate. Al-Ruddaf was found to be the richest site. It contained 6 species from the total identified fouteen species. The highest frequency of occurrence was recorded for *Agaricus bisporus*; *Lepiota procera* and *L. rhacodes* (Photo 2, 4 and 5, respectively). On the other hand, the rare existed species were *Lepiota cristata*, *Pleurotus ostreatus* and *Boletus edulis* (Photo 6, 7 and 14, respectively).

Sised locality was found to be the second site, it contain four species named *Pleurotus ostreatus*, *Coprinus comatus*, *Podaxis pistillaris* and *Phaeolepiota aurea* (Photo 7, 9, 11 and 13, respectively). The third locality was El-Shafa, it contain two species. The highest frequency of occurrence was recorded for *Inocybe splendens* and the lowest one was *Coprinus comatus* (Photo 12 and 9, respectively).

On the other hand only one species was recorded on the other tested localities. *Agaricus arvensis* was found in Al-Mathnah (Photo 1). *Lepiota cristata* was recorded on Sad Akrama (Photo 6), *Agrocybe cylindracea* was found on Wadi Mihrim (Photo 10) and *Agaricus augustus* was recorded on Al-Hada site (Photo 3).

Some of these species were reported before by several workers. In Egypt, Assawah [3] reported *Agrocybe* spp., *Hebeloma* spp., *Lepiota* spp. and *Tricholoma* spp. Also Abu El-Souod *et al*. [12] reported thirteen species of mushrooms belonging to ten genera. These genera were *Agrocybe*, *Armillaria*, *Coprinus*. *Drosella*, *Hebeloma*, *Hygrophorus*, *Lepiota*, *Leptonia*, *Panaeolus* and *Tricholoma*. In spain, Garcia *et al*. [22] and in Poland Falandysz *et al*. [23], identified *Coprinus campatus* while in USA. Richards [24] reported *Tricholoma* spp. Gray [25] reported that *Agaricus campastris* is common wild mushrooms in Europe and America.

Hayes [26] reported that natural geographic areas of mushrooms extends all over the northern Hemisphere outside the tropic and the arctic. Chin [27] recorded that twenty species of edible and poisonous mushrooms were collected from forests in Sarawak of the poisonous mushrooms were *Amanita excelsa*, *A. Phalloides*, *A. Pantherina*, *Clitocype.* sp. and *Nathopanus* sp. were included.

In China, edible mushrooms were also studied [13 - 15]. Also an *Agaricus Padanus* sp. was collected and identified in Italy [14]. Salzman *et al.* [28] used the neural signals to characterize the mushrooms.

Mushrooms can be used to solve several problems such as human nutrition and upgrade of waste products to be suitable for animal feed and hence avoiding waste pollution. So more studies should be carried out on mushrooms of Saudi Arabia.

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Genotypic Identification for Some *Fusarium sambucinum* Strains Isolated from Wheat in Upper Egypt

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Abstract: Random amplified polymorphic DNA (RAPD) was used to study the genetic variations among strains from *Fusarium sambucinum* isolated from wheat in Upper Egypt. Using two different primer (V6 and M13), the used strains showed a high degree of similarities and a distinct RAPD patterns. The dendrogram that constructed based on M13 primers showed that, there is no correlation between isolation sources and clustering system for the studied strains.

Key words: Phylogenetic dendrogram % phylogenetic % PCR % RAPD

INTRODUCTION

The taxonomy of *Fusarium* spp. is confusing and various classification systems have been proposed [1]. Species identification by morphological traits is problematic because characteristics like mycelial pigmentation, formation, shape and size of conidia are unstable and highly dependent on composition of media and environmental conditions. Phenotypic variation is abundant and many expertise are required to distinguish between closely related species and to recognize variation within species [2].

Some species of fungi need more experiences during their identification by classical methods. Right now with this revolution in the molecular techniques by using polymerase chain reaction (PCR) techniques, those problematic strains did not need so much effort to do identify well. Random PCR approaches are being increasingly used to generate molecular markers, which are useful for taxonomy and for characterizing fungal populations. Random amplified polymorphic DNA (RAPD) assay have been used extensively to define fungal populations at species, intraspecific, race and strain levels.

The use of molecular markers based on the polymerase chain reaction for species identification and as diagnostic tool became very popular during the last decade [3]. Once the primers are designed and conditions for a robust assay are optimized, PCR is very sensitive, rapid and relatively easy to handle assay. Welsh and McClelland [4] described RAPD-PCR technique for detecting genetic variation among different organisms. Genetic variability is assessed by employing short single primer of arbitrary nucleotide sequences. Specific sequence information of the organism under investigation is not required and amplification of genomic DNA is initiated at target sites which are distributed throughout the genome. Polymorphic fragments are the results of variation in the number of appropriate primer-matching sites of different DNAs. Nijs *et al.* [5] studied variation in random amplified polymorphic DNA patterns within *Fusarium* species from cereals from various parts of the Netherlands. Gherbawy [6] used RAPD technique to analyse different formae specials of *Fusarium oxysporum*. Möller *et al*. [7] studied fungal populations of *F. moniliforme* and *F. subglutinans* using RAPD technique. Gherbawy *et al*. [8] used RAPD technique for identifying of *Fusarium subglutinans*, *F. proliferatum* and *F. verticillioides* strains isolated from maize in Austria. Pasquali *et al.* [9] characterized isolates of *Fusarium oxysporum* pathogenic on *Argyranthemum frutescens* L. using RAPD technique.

An objective of the present study was to determine possible phylogenetic relationships among 15 representative strains of the species *Fusarium sambucinum* isolated from wheat in upper Egypt.

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Table 1: List of *Fusarium sambucinum* strains (isolated from wheat plants on two different types of media) used for RAPD-PCR analysis

Strains No.	*Fusarium sambucinum* strains	Sources of isolation	Media used for isolation
1	SVUML178	Rhizoplane	DCPA*
2	SVUML230	Rhizosphere	DCPA
3	SVUML255	Rhizosphere	DCPA
4	SVUML283	Rhizoplane	DCPA
5	SVUML300	Rhizoplane	DRBA**
6	SVUML303	Rhizoplane	DRBA
7	SVUML310	Soil	DCPA
8	SVUML320	Rhizoplane	DCPA
9	SVUML406	Soil	DCPA
10	SVUML420	Rhizoplane	DCPA
11	SVUML433	Soil	DRBA
12	SVUML440	Soil	DCPA
13	SVUML459	Soil	DCPA
14	SVUML491	Rhizoplane	DCPA
15	SVUML601	Rhizosphere	DCPA

DCPA* Dichloran chloramphenicol pepton agar
DRBA** Dichloran rose- bengal chloramphenicol agar

MATERIALS AND METHODS

Strains: Fifteen strains from *Fusarium sambucinum* were used in the present study. These strains were previously isolated from wheat plant in Upper Egypt by using dichloran chloramphenicol pepton agar (DCPA) and dichloran rose-bengal chloramphenicol agar (DRBA) media (Table 1).

DNA extraction: Fungal strains were cultured in 100 mL Erlenmeyer-flasks containing 20 mL Mandles Andreoti medium (per liter 10 g glucose; 2 g peptone; 2.8 g ammonium sulphate; 4 g KH_2PO_4; 10 g Na_2HPO_4; 10 mL of a simplified Czapek's conc.; 7 g $MgSO_4$; 0.05 g $CuSO_4$ $5H_2O$; 0.1 g $FeSO_4$ 7 H_2O; 0.1 $ZnSO_4$ 7 H_2O; final pH adjusted to 5) for 5 days using a rotary shaker (30°C, 150 rpm). The mycelium was collected by filtration and ground to fine powder in liquid N_2. Fifty milligram of the ground was transferred to a 1.5 mL Eppendorf tube and mixed with 0.7 mL 2 x CTAB buffer. Eppendorf tubes were incubated at 65°C for 30 min, then 0.7 mL of chloroform was added and mixed briefly. After centrifugation at 15.000 rpm for 30 min, the supernatant was transferred into a new tube mixed with 0.6 mL isopropanol and chilled to 20°C, followed by another centrifugation step for 5 min at maximum speed. The supernatant was discarded and the remaining pellet was twice washed with 1 mL of 70% ethanol, followed by drying under vacuum and thereafter dissolved in 1 mL TE (10 mM Tris, 1 mM EDTA, pH 7.5) buffer. DNA concentration were evaluated by agrose gel electrophoresis [10].

RAPD analysis: PCR conditions and separation of RAPD-PCR fragments were done according to the techniques of Messner *et al*. [11]. PCR's were carried out with the aid of primer V_6(5'DTGCAGBGTGG; [12]) and M_{13} (GAGGGTGGCGGT-TCT; [13]). PCR amplifications were performed in 50 µL volumes containing 1-1.5 unit Taq DNA polymerase (Biotherm, Gene Craft, Germany) dNTP mix (0.2 mM each of dCTP, dGPT, dATP and dTTP); 20 mM Tris-HCl (pH 8.4); 50 mM $MgCl_2$; 0.5 mM primer and 15-20 ng of genomic DNA. Amplification was performed in a thermalcycler (Flexigene, Techne, Cambridge, UK) with the following temperature profiles: 98°C for 5 min to denature genomic DNA. There were 40 cycles at 98°C for 15 sec; annealing at 40°C for 90 sec and extension at 72°C for 100 sec, followed by an additional cycle at 72°C. the PCR product were resolved by electrophoresis on 1.4% agarose gel in 0.5 X Tris-Borate-EDTA (TBE) buffer, at 125 V for 2 h. Gels were stained with ethidium bromide and photographed under UV light using UVP BioImaging CDS 8000 system (UVP).

RAPD data analysis: Computer analysis of RAPD patterns were performed as described by Halmschlager *et al*. [14], in which the band pattern obtained from agarose gel electrophoresis was digitalized by hand to a two discrete-character-matrix (0 and 1 for absence and presence of RAPD-bands, respectively). The analysis data was based on the Nei and Lee Coefficient [15]. Dandrograms were constructed by the unweighted paired group method of arithmetic average (UPGMA) based on Jaccard's similarity coefficient by using Phoretex ID software (version 5.2).

RESULTS AND DISCUSSION

Two different primers were used V6 (5'dTGCAGCGTGG; [12]) and M13 (5' dGAGGGTGGC GGTTCT; [13]) to analysis genetic variations among 15 strains of *Fusarium sambucinum*. The used primers (V6 and M13) in this work generated a considerable number of amplification products for comparison. Comparison of each profile for each of primers was based on the presence (1) versus absence (0) of RAPD amplimers that migrated to the same position in the gel. Bands of the same size obtained by the same primer were scored as identical, but only bands repeatable in at least two experiments with the same primer at different times were evaluated. All random primers resulted in robust RAPD fragment patterns (Fig. 1 and 2). All the used primers revealed a high degrees of similarities among

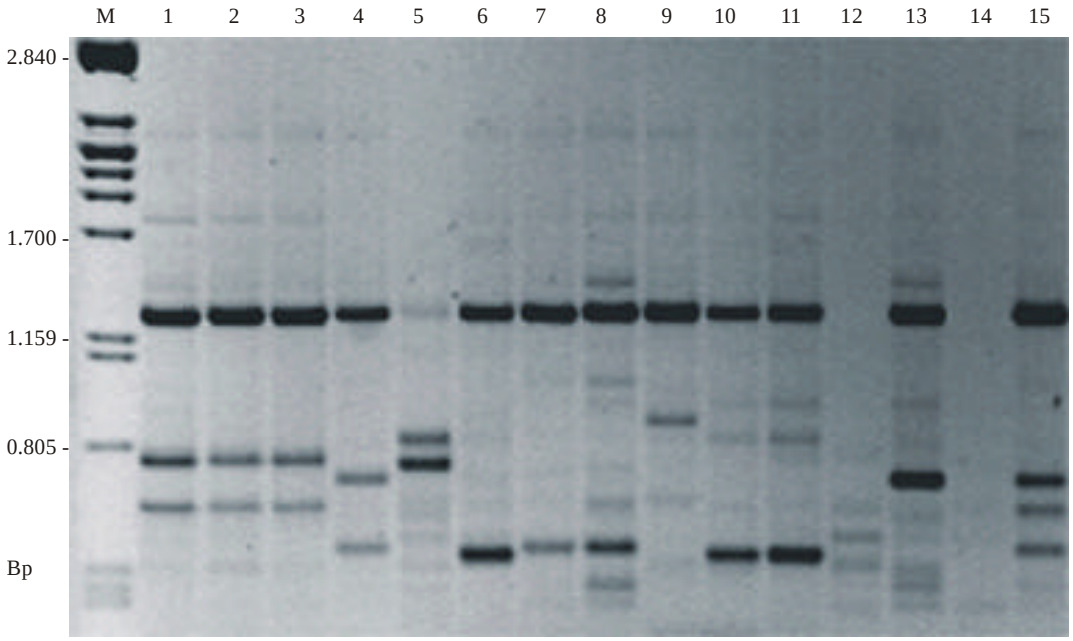

Fig. 1: DNA banding patterns from random amplified polymorphic DNA analysis of *Fusarium sambucinum* isolates primed by V6 (5'dTGCAGCGTGG; [12]). Lane M is a 100 Kb DNA

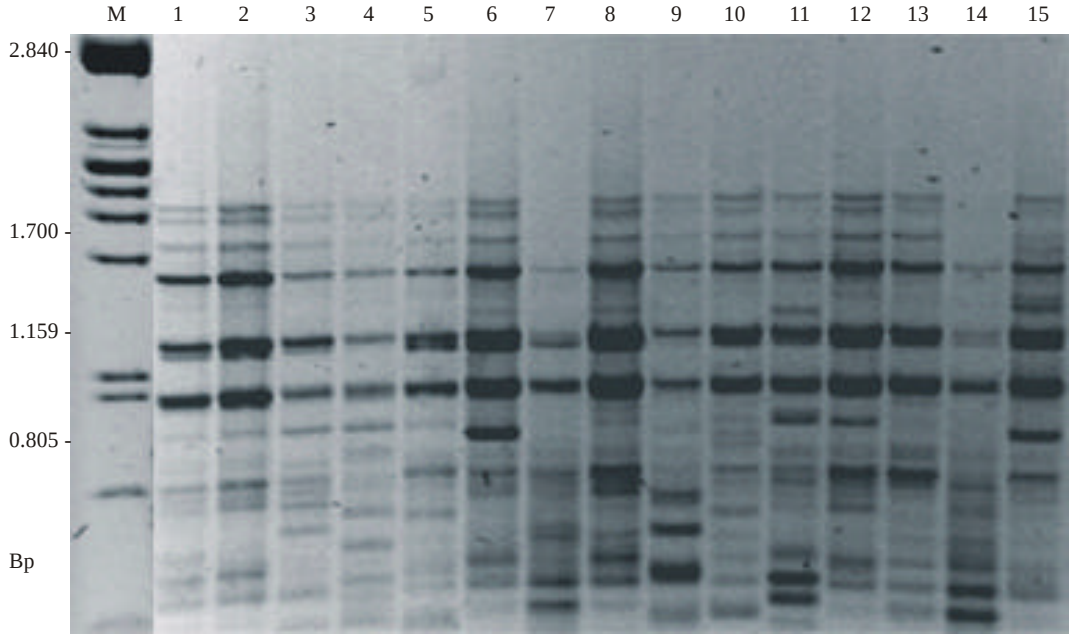

Fig. 2: DNA banding patterns from random amplified polymorphic DNA analysis of *Fusarium sambucinum* isolates primed by M13 (5' dGAGGGTGGCGGTTCT; [13]). Lane M is a 100 Kb DNA

Fusarium sambucinum strains (Fig. 1 and 2). Most amplification products were reproducible.

The RAPD data from M13 primer were used to construct a dendrogram. The dendrogram showing the relationship among the studied strains. This dendrogram showed that there is little correlation between some clusters of *Fusarium sambucinum* and isolation media, for example strains SVUML 255 (from rhizosphere and isolated on DCPA), SVUML 230 (from rhizosphere and isolated on DCPA) and SVUML 178 (from rhizoplane and

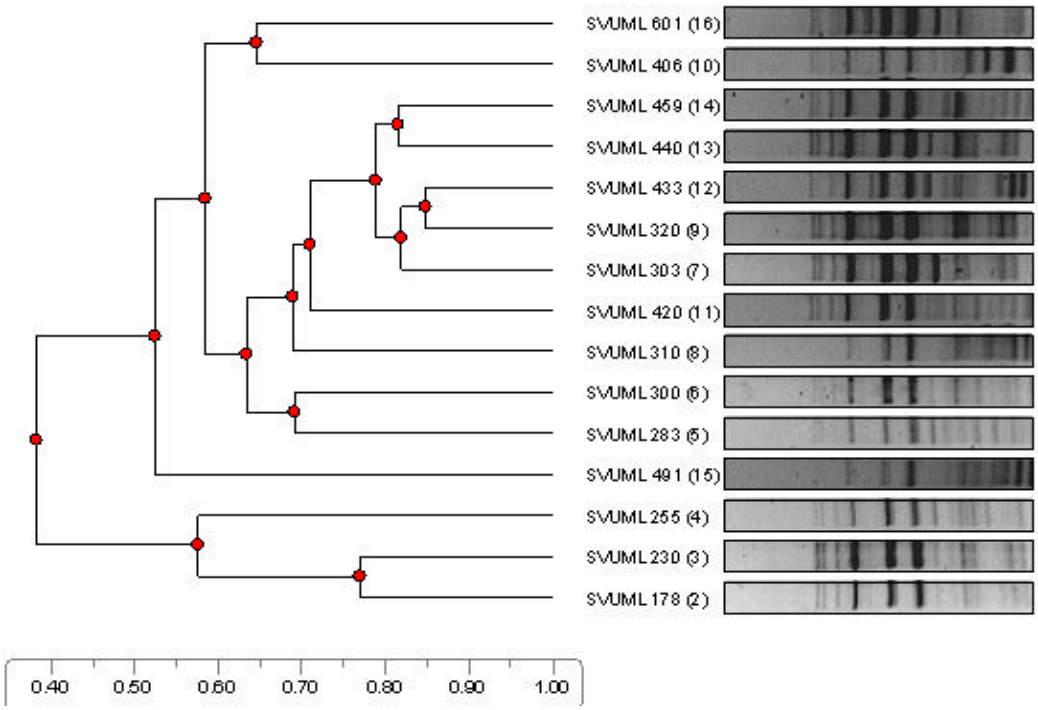

Fig. 3: Dendrogram showing relationships among 15 strains of *Fusarium sambucinum.* Genetic distances were obtained by random amplified polymorphic DNA analysis using M13 primer

isolated on DCPA) were clustered together in one group Fig. 3. In the other hand strains SVUML 300 (from rhizolane on DRBC) and SVUML 283 (from rhizoplne DCBA) clustered together. *Fusarium sambucinum* dendrogram indicated a little correlation between some clusters of *Fusarium sambucinum* and isolation media. DNA polymorphisms generated by the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) were used to analyse 41 isolates investigated in the European *Fusarium sambucinum* Project (EFSP) by Hering and Nirenberg [16]. They were employing ten arbitrary (10-mer) oigonucleotides and simple repeat sequences (M13, (GACA)4) as single primers, informative banding patterns typical for identifying European populations of *Fusarium sambucinum* Fuckel s. str., *F. torulosum* (Berk. and Curt.) Nirenberg and *F. venenatum* Nirenberg were obtained by them. Sixty seven authentic isolates, representing six species from *Fusarium* section *Fusarium* (= section Discolor) were subjected to random amplified polymorphic DNA (RAPD) analysis and polymerase chain reaction using species-specific primers by Wendy and Christianson [17]. They obtained remarkably uniform RAPD banding patterns intraspecifically, irrespective of the geographical origin of the isolates or the host/substratum from which they were isolated. Their molecular and morphological data support the identification of the Quorn strain as *F. venenatum* Nirenberg (= *F. sambucinum* Fuckel*sensu lato*).

The present study has shown that there is considerable genotypic variability among the Egyptian strains of *Fusarium sambucinum* obtained from different isolation source on different types of media.

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Variation in Germination and Ion Uptake in Barley Genotypes under Salinity Conditions

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Abstract: In this paper twelve barley (*Hordeum vulgare* L.) genotypes were screened for salt tolerance during seed germination in the Crop Production Department, Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordan. To evaluate salt tolerance during germination, 30 seeds of each genotype were placed on towel paper in 9 cm Petri dishes containing 20 mL distilled water or 1:1 M ratio NaCl and $CaCl_2$ solutions at various concentrations [(control), 100, 200, 300 mM] to give electrical conductivities (EC) of 0.05 (control), 10.6, 19.0 and 27.0 dS m^{-1}, respectively. Our data indicated that salinity level × genotype interaction effects ($p<0.05$) were observed for seed germination percentage, seed viability and ion uptake. Seed germination decreased significantly by increasing salinity level. Germination was significantly diminished at the highest level of salt (300 mM) with significant variation among genotypes and the genotype ACSAD1430 had higher germination percentage than other tested genotypes. Results presented in this article also indicated that the increasing seed pretreatment duration by hyper-saline medium significantly reduced seed recovery when transferred to distilled water. Also our data indicated that the increasing seed pretreatment duration by hyper-saline medium significantly reduced seed recovery when transferred to distilled water. The Na concentration of seeds after imbibitions significantly increased with increasing salinity with a considerable variation among genotypes. K concentration also affected by salinity. Generally, increasing salt stress significantly decreased K concentration in barley seeds after one day of imbibitions. The present study indicated that salt stress must be removed from soil surface for successful seedling establishment.

Key words: Barley % salinity % genotype % Jordan % germination

INTRODUCTION

Soil salinity is a major factor limiting plant productivity, affecting about 95 million hectares worldwide [1]. The UNEP (United Nations Environment Program) estimates that 20% of the agricultural land and 50% of the cropland in the world is salt-stressed [2]. Salinity imposes serious environmental problems that affect grassland cover and the availability of animal feed in arid and semi-arid regions [3]. Greenway and Munns [4] reported that some crops are moderately tolerant of saline conditions; many crops are negatively affected by even low levels of salt. Salt stress unfavorably affected plant growth and productivity during all developmental stages [5]. For example Epstein *et al*., [5] reported that salinity decreases seed germination, retards plant development and reduces crop yield. Seed germination is defined as the emergence of the radical through the seed coat [6]. Othman [7] reported that seed germination can be initiated by water imbibitions and any shortage in water supply will let seed under stress. Shokohifard *et al*., [8] reported that salt stress negatively affected seed germination; either osmotically through reduced water absorption or ionically through the accumulation of Na and Cl causing an imbalance in nutrient uptake and toxicity effect. Further, Younis *et al*., [9] reported that low moisture content under salt stress caused cessation of metabolism or inhibition of certain steps in metabolic sequences of germination. Conversely, salt stress increased the intake of toxic ions which may altered certain enzymatic or hormonal activities of the seeds during germination [10]. The barley is an example of a salt

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tolerant species [11], with genotypes that can germinate in seawater (i.e., about 47.0 dS m^{-1}) [12]. Barley is the most widely grown cereal crop in Jordan and other West Asian countries. The barley-based farming system exists in wide areas along the dry margins (200-300 mm rainfall per year) of cultivation in Syria, Jordan and Iraq [13, 14]. It is grown mainly as feed for livestock. Barley is considered highly salt tolerant of the agriculturally important cereals and has been grown successfully in fields that irrigation has rendered unsuitable for other crops [15]. Generally, barley grown near soil surface where the salts accumulated and at this point of soil, the concentration of salt change over time by continuous evapotranspiaration gradually rising salt levels or rainfall leaching salts from the soil surface supplying water to seeds [16, 17]. Variation in salt levels may restricted seed germination and in some cases resulting in the death of seeds [18, 19]. Germination of barley genotypes under different levels of salt stress and the ability of barley seeds to germinate after an extended period of exposure to salinity is unclear. Thus, the objectives of this study were to screen twelve barley genotypes for salt tolerance during germination, to study the ability of seed to germinate after exposure to different duration times of salt stress and to determine the ionic differences under salt stress.

MATERIALS AND METHODS

Three distinct experiments were conducted in the Crop Production Department, Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordan.

Plant material: Twelve barley genotypes (ACSAD176, WI-2291/ ER/Apm, SLB-6, Roho/A.Abiad, ACSAD1430, Esp//808/harmel, Aths/lignee68, JLB70-01, RUM, Mari/Aths/Attiki, ACSAD1212, ER/APM/3/) were used. Seeds of these genotypes were provided by the National Centre for Agricultural and Technology Transfer (NCARTT), Amman, Jordan.

Experiment 1: To evaluate salt tolerance during germination, 30 seeds of each genotype were placed on towel paper in 9 cm Petri dishes containing 20 mL distilled water or 1:1 M ratio NaCl and $CaCl_2$ solutions at various concentrations [(control), 100, 200, 300 mM] to give electrical conductivities (EC) of 0.05 (control), 10.6, 19.0 and 27.0 dS mG^{1}, respectively. Seeds were incubated in the dark at 22±1°C in completely randomized design arrangement with four replicates. Germination counts were made daily. Each petri dish was marked to indicate the solution level that must be maintained over the experimental period. Distilled water was added to each Petri dish as needed to maintain salt concentrations near target levels. Seeds were considered germinated when the radicle was at least 5 mm long. The experiment ended after about 10 days of incubation when no further seeds germinated for three successive days. Germination percentage was calculated using the equation:

Final germination percentage =
number of germinated seeds/ total number of seeds planted × 100

Experiment 2: In this experiment, the effects of salt duration pretreatment on seed viability were evaluated 30 seeds of each genotypes (replicated 4 times) were incubated with 1:1 M of NaCl and $CaCl_2$ solution (EC = 85 dS mG^{1}) for 1, 3, 5 and 7 days at 22°C in the dark. Seeds of each treatment and replicate were rinsed with distilled water then, transferred to Petri dishes containing distilled water. Seeds were counting daily and final germination percentages were determined as mentioned in experiment 1.

Experiment 3: In this experiment, ionic differences were evaluated. Seeds of all barley genotypes were incubated as mentioned in experiment 1. Seeds were soaked in distilled water or in salt solution in 50 mL glass containers. Three replicates of 30 seeds in 25 mL of solution were used for each soaking in solutions. After 24 h of incubation, seeds were removed from the container, dried to a constant dry weight at 80°C, ground to pass a 0.5 mm sieve in laboratory mill. The ground materials were mixed thoroughly and samples of 1.0 g were ashed for 5 h at 550°C in a muffle furnace, then the ash was dissolved in 2 M HC1 for determination of K and Na [20]. K and Na concentrations determined using flame photometer (Ion3).

Statistical analysis: Data were statistically analyzed using analyses of variance (ANOVA) using the MSTATC program (Michigan State Univ., East Lansing, MI and USA). Probabilities of significance among treatments and interaction and LSDs (p#0.05) were used to compare means within and among treatments.

RESULTS AND DISCUSSION

Effects of salt stress on seed germination: Analysis of variance revealed that significant differences among the barley genotypes for germination percentage. Salinity level × genotype interaction effects ($p<0.05$) were

Table 1: Germination percentage of barley genotypes as influenced by salinity levels after 10 days of incubation

	Salt level (mM)			
Genotypes	0	100	200	300
Roho/A.Abiad	84.5 ¶	57.9	33.3	3.7
ACSAD1212	82.3	65.0	51.7	26.7
Esp//808/harmel	85.8	64.0	34.2	16.7
SLB-6	84.3	47.4	50.8	25.8
Mari/Aths/Attiki	86.0	44.0	36.7	25.0
Aths/lignee686	81.8	42.5	60.8	26.7
RUM	85.5	64.2	23.3	25.0
ACSAD1430	84.3	86.3	68.3	41.7
ACSAD176	83.3	63.3	50.0	27.5
WI-2291/ER/Apm	83.0	57.9	51.8	24.9
JLB70-01	84.0	84.5	47.5	26.7
ER/APM/3/	84.3	65.8	52.9	25.8
LSD (0.05)		8.4		

Table 2: Germination percentage of barley genotypes as influenced by high level of salinity after 1, 3, 5 and 7 days of incubation

	Duration			
Genotypes	1 day	3 day	5 day	1 week
Roho/A.Abiad	62.3 ¶	21.3	4.25	2.25
ACSAD1212	48.0	50.8	74.50	16.30
Esp//808/harmel	70.3	52.3	35.00	6.50
SLB-6	41.0	30.0	18.00	13.50
Mari/Aths/Attiki	59.5	66.5	34.80	16.50
Aths/lignee686	68.3	61.0	42.00	26.50
RUM	61.0	56.5	30.30	8.50
ACSAD1430	89.8	85.0	60.30	5.50
ACSAD176	76.5	58.3	33.80	23.50
WI-2291/ER/Apm	54.3	42.8	18.30	6.50
JLB70-01	64.5	62.3	49.50	15.80
ER/APM/3/	60.0	41.5	17.50	5.50
LSD (0.05)		2.7		

Table 3: Sodium (Na) concentration (mg gG^{1}) of barley seeds soaked in distilled water and salt solutions

	Salt level (mM)			
Genotypes	0	100	200	300
Roho/A.Abiad	0.9 ¶	1.0	2.3	3.1
ACSAD1212	1.2	2.2	3.4	5.5
Esp//808/harmel	0.9	2.3	3.5	4.4
SLB-6	1.0	2.3	4.4	4.5
Mari/Aths/Attiki	1.0	3.5	3.4	3.8
Aths/lignee686	1.1	2.3	3.5	5.6
RUM	1.1	2.3	3.4	4.6
ACSAD1430	1.0	2.2	3.3	5.4
ACSAD176	1.0	1.5	4.5	6.1
WI-2291/ER/Apm	1.0	2.8	3.6	4.6
JLB70-01	1.2	4.4	4.1	4.3
ER/APM/3/	1.0	2.3	3.8	4.8
LSD (0.05)		0.50		

observed for seed germination (Table1). Interactions show that differences between genotypes depended on the salinity level. Germination percentage of barley genotypes was strongly affected by all salinity levels. Increased salt concentration caused a decrease in final germination percentage. Germination was greatly reduced at the highest level of salt (300 mM). Considerable variation among genotypes in response to salinity was observed for germination percentage. At 100 mM the genotypes ACSAD1430 and JLB70-01 had the highest germination percentage while at 200 and 300 mM salt the genotype ACSAD1430 had higher germination percentage than other tested genotypes. These results were in agreement with Basalah [21] who found that high levels of soil salinity can significantly inhibit seed germination. Further, Waisel [22] found that increasing salinity concentrations in germination often cause osmotic and/or specific toxicity which may reduce or retard germination percentage. Salt induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity [23].

Effect of salt duration pretreatment on seed viability: For this experiment salinity level × genotype interaction effects ($p<0.05$) were also observed for seed germination (Table 2). Interactions show that differences between genotypes depended on the salinity level. This variation possibly due to genetic variability among genotypes [24, 25]. During this experiment, seed pretreatment with salt solution for 1, 3, 5 and 7 days had adverse effect on germination percentage since no seed germination was observed. Xue *et al*., [26] reported that salt stress may affected seed germination through its toxicity effect by increasing Na and Cl concentration or by effects on the concentration and uptake rates of mineral nutrients. Recovery of germination was observed when transferred to distilled water. Increasing duration time of seed pretreatment by salt significantly reduced the germination percentage (Table 2). Based on the order of decreasing salt tolerance for seed germination after salt pretreatment (with their means overall duration pretreatment), the genotype ACSAD1430 had higher germination percentage than other genotypes while Roho/A.Abiad had the lowest germination percentage (Table 2). These results are in agreement with those reported by several researchers [9, 18, 27, 28].

Effects of salt stress on ion uptake: Analysis of variance revealed that significant differences among the barley genotypes for ion uptake. Our results also indicated that ion uptake was strongly affected by all salinity treatments. For example; seed Na concentration significantly increased with increasing salinity level for all studied genotypes (Table 3). At 100 mM, the genotype JLB70-01 had higher Na concentration than other genotypes. At 200 mM, the genotypes ACSAD176 and JLB70-01

Table 4: Potassium (K) concentration (mg gG¹) of barley seeds soaked in distilled water and salt solutions

	Salt level (mM)			
Genotypes	0	100	200	300
Roho/A.Abiad	5.1 ¶	4.2	3.3	3.3
ACSAD1212	6.6	3.8	4.0	4.1
Esp//808/harmel	5.9	3.5	4.1	2.7
SLB-6	6.8	4.0	4.9	3.9
Mari/Aths/Attiki	6.1	5.1	4.2	3.6
Aths/lignee686	5.5	4.9	3.4	4.2
RUM	4.7	3.5	4.4	3.6
ACSAD1430	8.0	3.3	5.5	3.1
ACSAD176	5.0	4.2	4.0	2.6
WI-2291/ER/Apm	5.0	3.3	3.4	3.5
JLB70-01	5.5	4.1	3.8	3.9
ER/APM/3/	5.9	4.3	3.8	3.1
LSD (0.05)		0.76		

had the highest Na concentration. On the other hand, at 300 mM the genotype ACSAD176 had higher concentration of Na than other genotypes.

Potassium concentration also significantly affected by salt stress (Table 4). Generally, K concentration decreased by increasing salinity (Table 4). Bhivare and Nimbaker [29] found that the reduction of K content and the increased of Na content in plant could be attributed to the effect of competition between Na and K ions on the absorptive sites of the plant. The reduction in K concentration causes a growth reduction by decrease the capacity of plants for osmotic adjustment and turgor maintenance or by the negative effects on metabolic functions as protein synthesis [4].

At 100 mM the genotype Mari/Aths/Attiki had higher K concentration than other genotypes while at 200 mM the genotype ACSAD1430 had the highest K concentration. In saline environment, high concentration of Na and Cl are usually the most injurious and predominant salts [30]. High level of Na caused a direct damage in plant cell membranes [31]. Haq *et al*., [32] found that Na concentration increased significantly with an increase in salinity from 1.2 to 15 dS mG¹ and this increase was 13.3 fold as compared to Na in plants grown under nonsaline conditions. Meanwhile Na concentration increased in barley seeds soaked in salt solution, seed germination decreased either by (a) increasing salt level or by (b) increasing duration time. This result support the hypothesis that Na increment inside plants had a toxic effects on seed germination, mainly by affecting the plant water relations or through displacement of Ca by Na from critical cell wall binding sites which could disrupt cell wall synthesis and hence inhibit plant growth [26, 33, 34].

In conclusion our result indicated the results of this study demonstrate that salt tolerance during germination exists within barley genotypes which represent a genetic material for development of salt tolerance of barley.

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Significance of Mycorrhizae

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INTRODUCTION

Mycorrhiza is a mutualistic association between fungi and higher plants [1]. Different types of mycorrhizae occur, distinguished by their morphology and to a certain extent, in their physiology. These include the ectomycorrhizae and endomycorrhizae. The ectomycorrhizae characterized by an external sheath of fungal cells surrounding the root, often penetrates between the cells of epidermis and the first few cells of cortex and the fungal hyphae typically infect the roots of forest trees of the temperate region. While endomycorrhizae like vesicular arbuscular mycorrhizal (VA) fungi forms no sheath, the fungus infects the root system of most cultivated crops and usually it invades several layers of the outer root cortex. VA-fungal hyphae penetrate individual cells and form arbuscules within the cell and vesicles outside their host cells which led to their name [2].

Vesicular-arbuscular mycorrhizal (VA) fungi colonize plant roots and ramify into the surrounding bulk soil extending the root depletion zone around the root system. They transport water and mineral nutrients from the soil to the plant while the fungus is benefiting from the carbon compounds provided by the host plant. Therefore VA-fungi have a pervasive effect upon plant form and function [3]. Little is known about the natural ecology of these fungal-plant associations and the effects of certain soil amendments with natural waste products.

VA-fungi are associated with improved growth of many plant species due to increased nutrients uptake, production of growth promoting substances, tolerance to drought, salinity and transplant shock and synergistic interaction with other beneficial soil microorganisms such as N-fixers and P-solubilizer [4]. Symbiotic association of plant roots with VA-fungi often result in enhanced growth because of increased acquisition of phosphorus (P) and other low mobile mineral nutrients [5]. Effective nutrient acquisition by VA-fungi is generally attributed to the extensive hyphal growth beyond the nutrient depletion zone surrounding the root [2]. Although a lack in growth response to VA-fungi inoculation in unsterilized soil was also recorded, this result has been attributed to the fact that native VA-fungi may provide the potential benefit of this mutualistic association [6].

It was reported that one of the principal avoidance strategies of plants for adaptation to adverse soil conditions is an increase in root surface area via mycorrhizae [7]. A better understanding of the mycorrhizae of agronomic crops is needed because of their potential involvement in systems of sustainable agriculture [8].

SIGNIFICANCE OF VA MYCORRHIZAE

Mineral nutrition:

Phosphorus: The major role of VA-fungi is to supply infected plant roots with phosphorus, because phosphorus is an extremely immobile element in soils. Even if phosphorus was added to soil in soluble form soon, it becomes immobilized as organic phosphorus, calcium phosphates, or other fixed forms [9, 10]. VA-fungi are known to be effective in increasing nutrient uptake, particularly phosphorus and biomass accumulation of many crops in low phosphorus soil [11]. Several investigators indicated that there is a beneficial effect of VA-fungi inoculation on nutrient uptake and on plant growth especially in sterilized soils [1, 12-14].

In white clover (*Trifolium repens* L.), mycorrhizal inoculation doubled the concentration of phosphorus in shoots and roots of infected plants and increased their dry weight [15]. Also Al-Karaki *et al*., [16] indicated that shoot dry matter, shoot phosphorus and root dry matter were higher for mycorrhizal infected wheat (*Triticum aestivum* L.) plants than for non infected plants. On the other hand, mycorrhizal infection has been shown to

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depress plant growth in soils with optimum phosphorus availability, these effect were attributed to competition for carbon between the host plant and the mycorrhizal fungi [11].

Nitrogen and micronutrients: The enhanced effect of VA-fungi on the uptake of nitrogen and micronutrient uptake may be attributed to two situations. In the first one is mycorrhizal hyphae act as extension to plant root, increasing root surface area and exploring larger soil volume, which will increase the chance of more micronutrient uptake. Mycorrhizal association with plant root may also enhance translocation between root and shoot of the infected plant, hence enhancing the plant growth [11].

At low phosphorus-levels in soil, mycorrhizae substantially increases copper and zinc contents of the shoot. However, it was found in case of soybean (*Glysine max* L.), grown in high phosphorsu-levels soils, the mycorrhizae decreases copper and zink contents of infected plants [17]. Peanut (*Arachis hypogaea* L.) plants grown in sterilized soil without VA-fungi inoculation developed visible symptoms of phosphorus and zinc deficiency [18].

Water relationship: Although most of the work done with VA-fungi has concentrated on their effects in plant nutrition, there is an increasing interest also on drought resistance of mycorrhizal plants [19]. VA-fungi infection has been reported to increase nutrient uptake in water stressed plants [20], enable plant to use water more efficiently and to increase root hydraulic conductivity [21]. Few studies however are available on the effect of water-stress on the fungi themselves, displayed by the number of spores in the soil and the root infection percentage.

Protection against toxic metals and pathogen: Few investigations were made about the importance of endomycorrhizal and ectomycorrhizal fungi in protecting host plants from phytopathogens and mineral elements toxicity. Still it was indicated that ectomycorrhizal fungi protect trees from high concentrations of toxic heavy metals, because these tend to be accumulated and immobilized in the mycorrhizal sheath [9].

VA-MYCORRHIZAL ASSOCIATION WITH LEGUME CROPS

Legume crops are generally cultivated in poor environments, even recently bred cultivars are selected to grow in such a poor environment and associated with its Rhizobium and an associated microflora Legume crops have a high (P) requirement for nodule formation, nitrogen fixation and optimum growth. Mycorrhizal condition of legume crops found to increase its vegetative growth and seed yield in addition to improve nodulation on it's root system [17,18]. Nair *et al*., [22] reported that higher level of VA mycorrhizal infection was beneficial for plant growth of cowpea (*Vigna unguiculata* L.) under field condition.

Hamel and Smith [23] reported that mixture growth of both corn (*Zea mays* L.) and soybean plants was greatly enhanced when inoculated with mycorrhizal fungi. Although more N appeared to be transferred from soybean to corn when plants were mycorrhizal, growth enhancement was attributed mainly to a better phosphorus uptake by mycorrhizal plants. Jackson and Mason [9] found positive relationships among (P) availability, VA mycorrhizal infection and pod yield in groundnut (*Arachis hypogaea* L.). It was indicated that mycorrhizal colonization in several cowpea genotypes was host dependent and heritable [24]. Alloush [25] found that chickpea plants inoculated with mycorrhizal fungus *Glomus versiforme* had higher number of nodules, shoot phosphorus content, shoot dry weight and grain yield than uninoculated chickpea plants.

EFFECT OF SOIL AMENDMENT WITH ORGANIC WASTES ON MYCORRHIZAL COLONIZATION

The materials we refer as organic wastes are merely those which are not put to use in our existing technological system. Once we begin to use them, they will no longer be called wastes and if they are in demand, we may even seek to increase their production. Organic wastes are really resources out of place. Farmers historically have applied animal manure and human wastes to the land, both treated and untreated, for crop production. Animal and crop plant wastes are different in their chemical and biological composition depending on the source of the material. Kale *et al*., [26] found that mycorrhizae in roots of a summer crop was 2.85% in soil previously received chemical fertilizers compared to 10% in the soil with half the recommended dosage of chemical fertilizers and organic matter (OM) amendment. Inoculation with VA-fungus did not significantly affect seed yield of pea (*Pisum sativum* L.) plants in soil which is rich in OM and phosphorus. On the contrary, seed yield was significantly enhanced with VA-fungi inoculation in soil which is poor in OM and phosphorus [3].

In mycorrhizae treatments, sludge showed inhibition of the mycorrhizal infection. This inhibition was persistent and apparently due to suppression of mycorrhizal fungi by toxic levels of NH_4^+ [17]. Also, both VA mycorrhiza spore density and root colonization were found to be higher under wastewater irrigated oldfield soils than in non-irrigated [27].

Large quantities of olive mill by-products are obtained when oils are extracted after mechanical and chemical treatments of olive yields [28]. The olive milling industry by-products; solid portion known as (Jift) or the liquids called (Zebar) could be used as soil OM amendment as Jift material is a nitrogen rich organic waste [29]. Although there are high levels of phytotoxic compounds found in fresh Jift which may inhibit seed germination or reduce plant growth, it contains no chemical contaminates like heavy metals [30]. On the other hand, Al Sakit and Al-Momani [31] found a positive relationship between fresh Jift amendment, olive seedling growth and association with mycorrhizae. There are no previous reports about the influence of the olive mill by-products, jift and zebar on the VA-fungi and its ecology and significance to commercial legume crops.

EFFECT OF SOIL STERILIZATION AND FUNGICIDE TREATMENTS ON MYCORRHIZAL INFECTION

Although large number of experiments studied the effect of different sterilization methods on soil pathogenic fungi, little information were reported about their effect on useful soil fungi.

Fungicide treatment: The effects of biocide use on non target organisms, such as VA-fungi, are of interest to agriculture, since inhibition of beneficial organisms may counteract benefits derived from pest and disease control.

Most of the fungicides which have been used to study their effect on VA mycorrhizal fungi were found to be deleterious, but some were quite compatible with VA mycorrhizal fungi. Sreenivasa and Bagyaraj [4] were studied the effect of nine fungicides on root colonization with VA mycorrhizal fungi and indicated that reduction from 10 to 20% of root infection percentages were recorded when the recommended level of fungicides were used. While some fungicides were significantly increased the percentage root colonization at half the recommended level.

In an experiment studied the effect of different fungicides on VA-fungi infection and population, it was concluded that application of fungicide to soil reduced sporulation and the root length colonized by VA-fungus, although interaction of VA-fungi and fungicide were observed to be highly variable depending on fungus-fungicide combination and on environmental conditions.

Solarization treatment: Soil solarization was shown to be cost reducing, compatible with other pest management tactics, readily integrated into standard production systems and a valid alternative to preplant fumigation with methyl bromide [32]. It also reported that soil solarization induced better growth response in plants even when no pathogen is present in the soil [33].

In field experiment, it was reported that solarization of soil by covering it with transparent plastic sheets resulted in reduction or complete elimination of soil pathogens between 0 and 25 cm depth in soil covered for 30-60 days [34]. In other experiment it was observed that covering the soil with a clear plastic sheet resulted in complete elimination of endomycorrhizal fungi at 10 and 20 cm soil depths [35]. It was also reported that root nodulation, infection by mycorrhizal fungi and yield of cowpea were higher in plants grown in solarized soil when compared to control treatment without solarization [22]. Stapleton and DeVay [33] indicated that the beneficial response of plant growth to soil solarization might have resulted from the effects of better root nodulation, enhanced VA mycorrhizal association and the increased availability of some of the macro and micro nutrients in soil solution due to solarization.

Methyl bromide treatment: Although there was a grave environmental concern about the application of methyl bromide and it's toxicity to mammals, it is still recommended for soil disinfection. Great reduction or complete elimination of all living organisms in the soil after methyl bromide gas fumigation of soil is well documented [25, 32]. Soil disinfection by methyl bromide fumigation or steam is often used to eliminate soil-borne plant pathogens, but such treatments can reduce VA mycorrhizal fungi as well [1]. Several studies have indicated that plant stunting following soil fumigation treatments may be due to elimination of VA mycorrhizae [36, 37].

EFFECT OF SOIL FERTILITY ON MYCORRHIZAL INFECTION

Most authors report extensive colonization to occur mainly in plants growing in soils of low fertility [8, 38]. Field and greenhouse studies demonstrated that crops growing in nutrient-poor soils had higher levels of mycorrhizal colonization than crops growing in better

soils [38]. Vesicular- arbuscular mycorrhiza inoculation in combination with phosphorus increased dry and fresh shoot weight, leaf area and leaf number of strawberry compared to application of phosphorus alone [39].

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Effect of Soil Amendment with Olive Mill By-products under Soil Solarization on Growth and Productivity of Faba Bean and Their Symbiosis with Mycorrhizal Fungi

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Abstract: Field experiments were carried out at JUST agricultural research center during 1999-2000 growing season to evaluate the effects of soil amendment with olive mill by-products (Jift) on growth of faba bean and their symbiosis with VA fungi. Soil was amended with Jift at different levels (Jift: Soil; 0:10, 1:9, 2:8, 3:7 and 4:6) and exposed to solarization, methyl bromide and fungicide treatments. A split plot design with three replications was used, in which soil treatments (solarization, methyl bromide, fungicide and untreated control) were assigned to main plots and soil-Jift mixtures to sub plots. Our data indicated that the maximum seed yield (2943 kg ha^{-1}) was achieved under soil mixtures treated with fungicide, followed by those which treated with methyl bromide (2662 kg ha^{-1}) and untreated control (2343 kg ha^{-1}). When Jift was considered as main factor, seed yield was found to be increased as Jift level was increased in soil mixtures. Even so, seed yield (2861 kg ha^{-1}) at the highest Jift level (3:7) was not considerably different from the yield at the rate of 2:8 (2998 kg ha^{-1}). Phosphorus nutrition may be enhanced with Jift amendment remarkably for soils branded by low organic matter contents. Mycorrhizal fungi population and symbiosis with the legume crops could be increased when Jift was added to the soil, particularly under soil sterilization practices.

Key words: Jift % Mycorrhizal % Faba bean % Olive

INTRODUCTION

Large quantities of olive mill by-products are obtained when oils are extracted after mechanical and chemical treatments of olive yields [1]. In Jordan, olive tree planting was doubled in the last few decades. As will, olive milling by-products, mainly Jift, is expected to be increased accordingly. The amount of Jift produced is estimated to be over 100,000 ton per year. However, one of the viable solutions to benefit from these organic materials is to be used as soil amendment [2]. Farmers historically have applied animal manure and human wastes to the land to increase their productivity [3-5]. It was established that organic matter portion of the soil is very important to maintain soil physical and chemical properties to be optimum for crop production [6-12]. Recently, extensive application of chemical fertilizers is becoming of increasing concern to the environment and human health [13]. Therefore, using organic wastes like Jift as fertilizers may reduce the amount of chemical that applied to the soil. Despite of the fact that Jift is a nutrient rich organic waste, high levels of phytotoxic compounds present in fresh Jift, which may inhibit seed germination or reduce plant growth [14]. However, it was found that composting Jift may reduce its phytotoxicity compared to fresh one [14]. In the present study, the effect of Jift on faba bean was investigated by using different levels of Jift in soil mixtures to determine the optimum level that maximize the yield. The effects of soil amendment with organic matter on soil ecology, especially the beneficially microorganisms like vesicular arbuscular mycorrhizal fungi is very important before making any recommendations. However, there are no previous reports about the influence of the olive mill by-product (Jift), on the VA-fungi and its ecology and significance to commercial legume crops. VA is recognized as being of considerable benefit to the plant host, especially when the root system is not very extensive and soil nutrient status is low. It was indicated that mycorrhizal fungi may increase the uptake of many nutrients such as P, nitrogen (N), sulfur (S), calcium (Ca), zinc (Zn) and copper (Cu) [15]. Mycorrhizal fungi are

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found in association with the roots of almost all kinds of plants. Different types of mycorrhizal fungi are found, ectomycorrhiza and endomycorrhiza [16]. The ectomycorrhiza are typically infecting forest trees [17]. But endomycorrhiza like vesicular arbuscular (VA), usually infect most of the cultivated crops [18]. The fungus produces enlarged vesicles and clusters of branched hyphal ending (the arbuscules) giving us the name for this mycorrhiza type. The tendency to recommend the use of VAM fungi for improving the productivity of soil comes from that VAM fungi are most adapted to help plants in marginal and low productive soils [19]. Environmental conditions such as drought, salinity, mineral nutrition, pH and organic matter may influence the intensity of infection by mycorrhiza fungi on their proper host plants [20-22].

Increase soil organic matter will increase the amount of nutrients and the ability of the soil to make those nutrients available for plants [23-27]. Also it was observed that moderate deficiency of N, P and K promotes mycorrhizal formation [28]. Several studies indicated that soil amendment with OM increased mycorrhizal fungi infection [16,29]. Al-Sakit and Al-Momani [30] found a positive relationship between fresh Jift amendment, olive seedling growth and association with mycorrhizae. The purpose of this study was to evaluate growth and yield of faba bean grown in soil treated with olive mill by-products and also to determine the effect of olive mill by-products on soil mycorrhizal fungi population associated with faba bean crop.

MATERIALS AND METHODS

Site description: Field experiments were conducted at JUST research station in the northern part of Jordan [32° 34' N latitude, 36° 01' E longitude and 520 m altitude). Climate is Mediterranean, characterized by mild rainy winter and dry hot summer [31]. Total amount and distribution of rainfall for the period 1995-1999 are presented in Table 1. The soil is silty, clay loam [32]. Soil chemical characteristics are presented in Table 2.

Environmental condition: Temperature and rainfall were monitored during the growth period of faba bean crop. Faba bean plants were irrigated from flowering stage until physiological maturity stage with drip irrigation system to supplement rainfall and maintain soil moisture near field capacity. All plots were hand weeded as needed throughout the growing season.

Land preparation and experimental design: Two plowing operations were done by disk plow followed by disk harrowing to mix and levelling soil surface. Surface soil was levelled and the outside boundaries were layed down to give 2 × 1 m^2 land lot. The whole lot was made into 20 cm spaced rows with the aid of field machinery. An experimental plot consists of five rows of 2 m long, 20 cm apart and 50 cm space between those plots. This design gave a replicated treatment lots of 2 × 1 m^2 area.

Table 1: Amount of precipitation at Ramtha experimental station during the period 1989-1999

Growing seasons	Rainfall amount (mm)
89/90	248
90/91	160
91/92	379
92/93	201
93/94	182
94/95	274
95/96	161
96/97	245
97/98	272
98/99	108

Table 2: Analysis of soil at Jordan University of Science and Technology

Properties	Value
Soil texture	Silty clay loam
Soil pH	8.380
EC (ds cmG^1)	0.566
Organic matter (%)	0.927
Available P (PPM)	9.875
Total N (%)	0.243

The soil treatment methods (Methyl bromide fumigation, fungicide treatment and untreated control) were assigned to main plots. Each main plot was divided into four sub-plots that comprised of the four ratios of soil and Jift (J: S; 0:10, 1:9, 2:8 and 3:7).

Application of olive oil mills by-products: Soil was mixed with Jift at four different levels of soil: Jift (S: J); 10:0, 9:1, 8:2 and 7:3, on volume basis and for the first 15 cm of soil depth. Those levels were attained by adding 0 m^3, 0.015 m^3, 0.030 m^3, 0.045 m^3 of Jift per 1 m^2 of soil, respectively.

Soil treatments: Plots previously received proposed Jift levels in each experiment were fumigated with methyl bromide under air-tight plastic sheets for three days and the fumes were allowed to dissipate for 10 days. In another treatment similar plots were treated with a fungicide, or left untreated as a control. A systemic fungicide (a.i. Metalaxyl- 50 g kgG^1, obtained from Veterinary and Agricultural Products Co. Ltd.) was used. Four Kg per donum of the fungicide granules were mixed with the soil immediately before planting.

Planting: Faba bean (local cultivar) was sown on 16th January, 2000. The crop was seeded at 10 seeds per row. This will give plant densities of 25 plants per m^2.

Data collection:

Phonological traits: Time from planting to emergence and percent emergence were recorded when 90% of seedlings had just emerged from the soil. At physiological maturity stage, five plants were sampled randomly from each plot. The following parameters were recorded for each plant: plant height (cm), number of branches, number of leaves, number of pods and number of seeds. The average reading of the five plants was presented as per plant.

Yield and yield component: Harvesting was performed when each crop reached its physiological maturity. Crop yield per unit area were obtained by harvesting the three central rows (150 cm × 60 cm = 0.9 m^2). The outer two rows and 25 cm at both sides of the central rows were left to avoid border effects. Plants were manually harvested by a hand scissors, placed in open plastic bags, air dried for a week. Biological yield was determined by weighing the total dry matter of above ground parts including seeds and straw. The yield was converted into kg haG^1 basis. Grain yield were measured and converted to kg haG^1. Harvest index was calculated as the ratio of seed yield to biological yield multiplied by 100 [33].

Phosphorus analysis: Phosphorus concentration and content in plant tissue were determined at fruit sitting stage. Dried plant materials were finely ground using a scientific mill. Representative samples ranging from 0.5 to 1.0 g were taken and put into crucibles. Crucibles were placed inside muffle furnace at 550°C for at least 5 h after attaining the desired temperature for dry ashing. Ash contents of the crucibles were digested by adding 5 mL of 2 N HCl, agitated and left for 30 min. Crucibles contents were filtrated with Whatman No. 42 filter paper and then the filtrates were collected in small plastic bottles. P concentrations in filtrates were determined colorimetrically using the yellow method [34].

Determination of number of VAM spores in the soil: The method which used to isolate VAM spores in soil was the floatation-adhesive technique which adopted by Sutton and Barron [35]. This method included pulling representative soil samples from field plots and potted soil which is then placed inside closed plastic bag and stored inside cooled chamber at 20°C. Sub samples of 10 g from each soil samples of the replicated treatments was placed inside a 500 mL graduated cylinder and 150 mL of distilled water was added. The cylinder was strongly shaken for 10 min then left setting for 7-15 min for the heavy soil particles to settle down. The supernatant soil solution was transferred to 500 mL separator funnel and shaken for 2-3 min. Then the funnel was fixed to a vertical stand and left for 5 min. The solution inside the funnels was allowed to drain via the funnel valve at a rate of 75-100 drops per min. After the complete drainage of the solution, the internal surface of the separator funnel was washed with 3 to 5 mL of distilled water and collected on filter paper fixed inside normal funnel underneath. The filter paper was stretched inside a petri dish with the collected precipitates facing upward. VA spores collected on the filter paper were counted by placing four cover slips (4 cm^2) at random sites on filter paper using a compound microscope. Number of spores under each cover slip was counted. Total number of spores and different spore population was calculated according to the following formula:

Number of spores on the filter paper =
Total number of spores under the four cover slips × 3.975

Determination of VAM roots infection: Root samples were gathered at flowering and physiological maturity stage, stored inside cooled chamber at 4°C. Roots were cleared with 10% KOH and stained with 0.05% Trypan Blue using the procedure of Phillips and Hayman [36]. Determination of the extent of VA mycorrhiza colonization was done by estimating the percentage of the length of each segment which is colonized according to the method of Bierman and Linderman [37].

Statistical analysis: Data were analyzed with standard ANOVA technique using MSTATC PROGRAM (Michigan State University, East Lancing. Mich., USA). Least significant differences at the 0.05 probability level were used for the separation of treatment means.

RESULTS AND DISCUSSION

Effects of Jift in soil mixtures, soil treatments (methyl bromide, fungicide and control) and their interactions on faba bean plants were investigated throughout the life cycle of this crop under open field conditions and the following results were observed.

Faba bean seeds, cross treatments, attained 50 and 100% emergence at 22 and 26 days from the seeding date, respectively. Plants reached flowering stage at around 65 days after emergence. Fruit setting stage was attained around 90 days after 100% emergence. However, harvesting was done at 160 days from seeding date and when plants in all plots reached their physiological maturity stage.

Table 3: Effect of soil treatments (solarized, methyl bromide fumigated, fungicide treated and untreated control) & Jift rates on plant height, number of branches per plant, number of leaves per plant, number of pods per plant and number of seeds per plant of faba bean at physiological maturity stage in the field

Soil treatments	Mixture rates	Plant height (cm)	No. of branches per plant	No. of leaves per	No. of pods per plant	No. of seeds per plant
Methyl bromide	0:10	45.8	44.5	4.3	5.6	12.9
	1:9	54.7	59.7	3.9	6.9	15.7
	2:8	53.4	69.3	2.9	8.1	21.5
	3:7	54.7	69.9	2.9	8.9	21.3
Fungicide	0:10	50.4	44.9	3.6	6.5	17.2
	1:9	48.9	60.4	4.0	7.3	20.0
	2:8	58.9	69.2	3.6	8.3	20.5
	3:7	60.0	69.2	3.2	8.1	19.5
Control	0:10	45.0	46.3	2.9	5.6	14.6
	1:9	46.8	56.5	3.9	6.8	17.8
	2:8	50.9	62.9	4.7	6.7	18.3
	3:7	52.7	57.2	5.4	5.7	16.5
LSD (p#0.05)		4.7	5.5	1.6	1.1	3.2

Table 4: Effect of soil treatments (methyl bromide fumigated, fungicide treated and untreated control) on yield and yield components per area (kg ha^{-1}) of faba bean at physiological maturity stage in the field

Soil treatment	Seeds yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest indes (%)
Methyl bromide	2662	3230	5893	45.3
Fungicide	2943	3413	6356	46.6
Control	2343	2605	4949	47.5
LSD (p#0.05)	185	272	319	2.6

Table 5: Effect of Jift rates on yield and yield components per area (kg ha^{-1}) of Faba bean at physiological maturity stage in the field

Mixture rates (J:S)	Seeds yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (%)
0:10	2182	2334	4517	48.2
1:9	2556	3262	5817	44.0
2:8	2998	3253	6252	48.2
3:7	2861	3482	6344	45.3
LSD (p#0.05)	295	361	610	2.1

Yield and yield components:

Biological and straw yields: The highest biological yield was produced with fungicide (6356 kg ha^{-1}), followed by methyl bromide (5893 kg ha^{-1}) and control (4949 kg ha^{-1}) treatments. Straw yield showed the same trend, in which the highest value was obtained with fungicide (3413 kg ha^{-1}), followed by methyl bromide (3230 kg ha^{-1}) and control (2605 kg ha^{-1}) treatments (Table 4). There was an increase in biological and straw yields recorded as a consequence of Jift addition at the rate of 1:9. However, the addition of Jift at the rates of 2:8 and 3:7 had no significant adverse effect on biological and straw yields (Table 5). These results may be explained by the high level of phytotoxic compounds like phenols which is present in fresh Jift. Pages *et al*., [14] indicated that Jift contains high levels of phytotoxic compounds which may inhibit seed germination or reduce plant growth. Those results are in agreement with what was reported by Turk *et al*., [38] in which faba bean plants tolerated high levels of Jift in the rooting medium. Therefore its further documented that faba bean roots are able to tolerate the high amount of phenolic compounds present in Jift and this product could be used selectively against more sensitive weeds in faba bean cropping as a bioherbicide. Our results further substantiated the positive role of Jift in supporting the legume mycorrhizal association. That kind of effect was reflected by the increase in yield recorded in consequence of Jift addition at the rate of 1:9, although the faba bean yield not significantly affected by further Jift addition (Table 5). These results indicated the possibility of improved physical and chemical properties of the soil that achieved by Jift amendment. Increasing soil organic matter, especially rich nutrient organic matter like Jift, may increase the availability of most nutrients in the soil. Similar phenomenon were approved to be present by many researchers Turk *et al*., [38]. It was established that animal manure and other organic wastes improves soil chemical and physical properties.

Harvest index: Harvest index values were slightly higher for plants grown in plots left as untreated control than those grown in plots treated with fungicide or fumigated with methyl bromide. Faba bean plants grown in soil mixtures with the rates of 0:10 and 2:8 Jift to soil had higher harvest index than plants grown in soil mixtures with the rates of 1:9 and 3:7. However the main effect of soil treatments and their interactions with soil mixtures did not significantly alter the harvest index values significantly at p#0.05 (Tables 4 and 5).

Seed and pod numbers per plant: There was a tendency of increased pod and seed numbers per plant as Jift level was increased in soil mixtures, under fungicide and methyl bromide treatments. Similar trend was showed by plants grown under plots left as untreated control, but the highest level of Jift in soil mixtures (3:7) caused significant reduction in pod and seed numbers compared to the next lower level (2:8) (Table 3). These results confirm the findings of Tattini *et al*., [39], in which they have reported that yield of peach (*Prunus persica*) and olive (*Olea europaea*) were increased with soil application of olive mill waste materials.

Seed yield: Results showed that the highest seed yield (2943 kg ha^{-1}) was achieved under soil mixtures treated with fungicide, followed by those which treated with methyl bromide (2662 kg ha^{-1}) and untreated control

(2343 kg ha^{-1}) (Table 4). When Jift was considered as main factor, seed yield was found to be increased as Jift level was increased in soil mixtures. However, seed yield (2861 kg ha^{-1}) at the highest Jift level (3:7) was not significantly different from the yield at the rate of 2:8 (2998 kg ha^{-1}) (Table 5). The results of this study demonstrate the Jift application could be improve the soil physical properties including soil structure, water holding capacity and infiltration rate. In addition organic matter amendment may increase availability of most nutrients and provide energy for microbial activity [40]. Similar phenomenon was approved to be present by many researchers [38]. It was established that animal manure and other organic wastes improves soil chemical and physical properties.

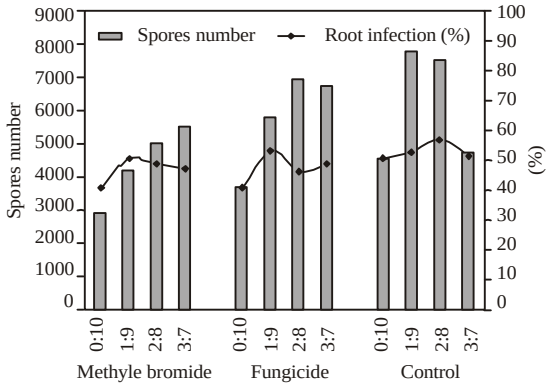

Fig. 1: Effect of soil treatments (methyl bromide fumigated, fungicide treated and untreated control) & Jift rates on number of spores per 10 g soil and root infection percentage of faba bean at fruit setting stage in the field

Phonological traits:

Plant height: Faba bean plants grown in soil mixtures containing Jift at all rates were taller than those grown in soil mixtures containing zero Jift, under all soil treatments This might be due to significant influence of jift on early establishment of faba bean plants and subsequent early growth. These results are in agreement with those reported by Carter *et al*., [9], who found that manure application have significant impact on early establishment of sorghum plants and subsequent vigorous growth as assessed by plant height measurement. Under methyl bromide treatment, increasing Jift level at the rates of 2:8 and 3:7 did not affect plant height significantly compared to Jift addition at the rate of 1:9. However, the height of faba bean plants was increased as Jift level was increased in soil mixtures treated with fungicide or left as untreated control. Irrespective to Jift levels, the tallest plants were observed under fungicide treatment over the other soil treatments (Table 3).

Leaf and branch numbers per plant: There was a tendency of increased leaf numbers as along as Jift level was increased in soil mixtures, even for the highest Jift level (3:7) under methyl bromide and fungicide treatments. Similar results were observed for soil mixtures that left as untreated control, but the highest Jift level (3:7) caused significant reduction in leaf numbers compared to the next lower level (2:8). The higher number of leaves associated with jif application may be due to more availability of P and N with consequently more rate of leaf appearance. This is in line with the findings of Nikus *et al*., [4]. Irrespective to Jift levels in soil mixtures, it was observed that plants grown in plots treated with fungicide or methyl bromide had higher leaf numbers than those grown in plots left as untreated control (Table 3). Number of branches was decreased with high Jift levels (2:8 and 3:7) in soil mixtures, under methyl bromide treatment. But it was not affected by Jift levels under fungicide treatment. However, branches number was increased along with increasing Jift level in soil mixtures left as untreated control (Table 3).

Mycorrhizal infection: VA spore numbers were increased as Jift level was increased in soil mixtures, under methyl bromide and fungicide treatments. Similar trend was observed in untreated control plots, but the highest Jift level (3:7) caused significant reduction in spore numbers compared to soil mixtures at the rates of 1:9 and 2:9 and its value was comparable to that obtained with zero Jift mixtures (Fig. 1). Such decrease in number of spores and root infection as a result of waste amendment were also indicated by several researchers [41]. Root infection percentages observed on roots of faba bean plants grown in untreated control plots were higher than what observed on plants grown in methyl bromide and fungicide treated plots. These results are in agreement with previous finding in previous investigations [42].There was an increase in root infection percentage in response to Jift addition at the rate of 1:9 in soil mixtures. However, significant response of root infection to Jift levels and their interactions with soil treatments were absent at p#0.05 (Fig. 1).

Phosphorus percentages and contents per plant: There was an increase in phosphorus percentages as Jift level was increased in soil mixtures, under plots treated with methyl bromide or left as untreated control. However,

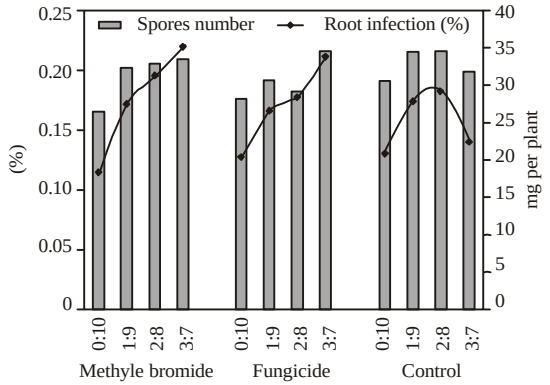

Fig. 2: Effect of soil treatments (methyl bromide fumigated, fungicide treated and untreated control) & Jift rates on P concentration and total P content per plant of faba bean at fruit setting stage in the field

only Jift addition at the rate of 3:7 increased phosphorus percentages of faba bean plant tissues under fungicide treatments.

Phosphorus contents per plant were increased as Jift level was increased in soil mixtures, under methyl bromide and fungicide treatments. Phosphorus percentages also, were increased in response to increasing Jift in soil mixtures, although the highest Jift level (3:7) were mostly produced phosphorus percentages comparable to that obtained under zero Jift mixtures (Fig. 2). Such relationship between organic matter content in the soil and shoot phosphorus percentages were previously observed by Turk *et al*., [38].

CONCLUSIONS

In countries like Jordan, where olive plantation is a major crop, the olive mill by-products are likely used as soil amendment for one purpose or more. Due to the fact that those products contain phytotoxic components and they are under uncontrolled disposal, farmers and growers conceived a negative attitude against their usage in agriculture as soil treatment. However, the present investigation demonstrated that the highest seed yield was observed under soil mixtures treated with fungicide, followed by those which treated with methyl bromide and untreated control. When Jift was considered as main factor, seed yield was found to be increased as Jift level was increased in soil mixtures. However, seed yield at the highest Jift level (3:7) was not significantly different from the yield at the rate of 2:8. our results confirm the findings of Turk *et al*., [38], in which they have reported that yield of wheat was increased with soil application of olive mill waste materials.

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Monitoring of Cultivars Identity and Genetic Stability in Strawberry Varieties Grown in Egypt

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Abstract: A simple and routine method for the analysis of tissue culture-derived strawberry plants for somaclonal variations is a prerquist for pricise monitring of quality control during rapid mass micropropgation. Similarly, molecular identification of different varieties is an important element for efficient and effective management of strawberry genetic resources. This study reports on the use of RAPD-PCR for identification of different cultivars of strawberry and detection of genetic variations in micropropagated strawberry plants. Seven varieties of the cultivated strawberry, grown in Egypt, were screened using RAPD-DNA markers. Only four RAPD primers (among 20 tested) were chosen as producing polymorphic DNA bands differentiating the investigated cultivars. Based on those identity markers, the genetic distances between varieties were determined and their genetic relationships were estimated. The phylogenetic tree revealed that the seven studied cultivars showed close similarity within the group. Although minor morphological variations were recorded in the leaves of some clones, the developed RAPD profiles of different micropropagated clones were typical to that of the donor mother plant.

Key words: RAPD markers % somaclonal variation % genetic similarity

INTRODUCTION

The strawberry (*Fragaria*) is a genus of plants of the family *Rosaceae*. The leaves typically have three leaflets, but the number of leaflets may be five or one. There are more than 20 *Fragaria* species worldwide. There are seven basic *types* of chromosomes that they all have in common. However, they exhibit different polyploidy [1]. Some species are diploid, having two sets of the seven chromosomes (14 chromosomes total). Others are tetraploid (4× = 28), hexaploid (6× = 42), octoploid (8× = 56), or decaploid (10× = 70).

Strawberry plants spread vegetatively using runners and this enables them to be easily transplanted and propagated as clones. Commercial production of strawberry using micropropagation processes bears several risks. Plant off-types, i.e. non true-to-type and genetically not identical to the mother plant, may be among the resulting plants. These plants can simply the result of hardening errors and not arise from a change in the genetic make up of the plant [2].

I*n vitro* production of plants involves the application of plant growth regulator, such as auxins, for process initiation. Nevertheless, these auxins are known to be associated with genetic instability in plants, a phenomenon called somaclonal variation [3-5]. Although somaclonal variation may be used as a source for variation to get superior clones, it could be also a very serious problem in the plant tissue culture industry resulting in the production of undesirable plant off-types [6, 7].

Since somaclonal variation was first defined [8], it has been widely documented in tissue culture-raised plants at morphological, chromosomal, biochemical and molecular levels in many plant species and extensively reviewed [9, 10]. Polymorphism at DNA level among the somaclonal families which phenotypically normal was reported in strawberry [11] in *Triticum* [12], in rice [13], in *Populus deltoids* [14] and in date palm [15, 16]. Such modifications included gene methylation changes, DNA rearrangements and alterations in copy number. However, it is sometimes hard to differentiate such permanent somaclonal variability from transient epigenetic changes. These epigenetic changes might include transient expressions or modifications of a certain plant trait. But in contrast to somaclonal changes, such trait is not passed to their offspring through the sexual cycle or might entirely disappear during plant maturation.

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Epigenetic changes are often demonstrated after an exposure of plant material to a stressful condition and may be due to DNA amplification, DNA methylation, or activation of transposable elements [17].

In order to evaluate genetic variability, follow phylogenetic origin and extent of ecologically distinguished species or subspecies and to develop efficient crop breeding systems, plant breeders need to have a definitive identification both of cultivars and selections of crop plants. Reliable methods of identification are also required for the establishment of plant variety rights [18]. Unambiguous identification is especially important in a clonally-propagated crop such as strawberry [19]. Commercial cultivators need to be sure that they are investing their time and money in propagating the specific cultivar that they have chosen on the basis of yield, harvest time, size and shape.

Presently, there are various methods available which can be used to detect and monitor tissue culture-derived plants and cultivar identification. The most reliable methods are the molecular marker techniques that identify the variance depending on the plant proteins, which are expressed from defined regions of DNA, or DNA polymorphisms. RAPD (random amplified polymorphic DNAs) is a powerful technique for identification of genetic variation [20]. It has the distinct advantage of being technically simple and quick to perform, requiring only small amounts of DNA compared to restriction fragment length polymorphism (RFLP) analysis [21].

Strawberries (*Fragaria* × *ananassa* Duch.) have been extensively analyzed using randomly generated markers for clone identification and diversity studies [22-27]. òebrowska and Tyrka [28] have used RAPD markers for strawberry identification and genetic diversity studies. The obtained results confirmed the usefulness of RAPD markers for strawberry cultivars identification, author's property rights protection and selection of parents suitable for creating of mapping population.

In the present study, the primary objectives were to use RAPDs to examine the genetic integrity and uniformity of the important strawberry cultivars and tissue culture-derived plants and to generate useful DNA fingerprints to facilitate cultivar identification. In addition, the genetic relationships that exist between these cultivars were examined as well as genetic stability was monitored.

MATERIAL AND METHODS

Plant materials: Seven commercially grown strawberry cultivars, namely Camarosa, Chandler, Osogrande, Kabetula, Selected, Rosalinda and Laguna, were used in this study. Different cultivars were secured from Strawberry Research Center, Faculty of Agriculture, Ain Shams University.

***In vitro* propagation of strawberry:** Shoot tips were separated from runners, sterilized and cultured onto the medium recommended by Boxus [29] supplemented with 6-benzylaminopurine (BA, 0.5 mg dmG^3), gibberellic acid (GA_3, 0.1 mg dmG^3), indole-3-butyric acid (IBA, 0.1 mg dmG^3), glucose (40.0 g dmG^3) and Bacto-Difco agar (6.4 g dmG^3). Afterwards, shoots were multiplied and subsequently rooted *in vitro* on the same medium without BA and GA_3. Obtained plants were transplanted to mixture of peat, perlite and sand (6:2:2 v/v, pH = 5.5) and fertilized with 1/2 strength MS salts.

Genomic DNA isolation: DNA of the 7 Egyptian strawberry cultivars and of different tissue culture-derived clones (cv. Rosalinda) was extracted from the leaves following the method described by Rogers and Bendich [30] with the modification that the fresh leaves were lyophilized and ground to fine powder in a swinging ball mill MM 2000 (Retsch GmbH, Haan, Germany).

RAPD-PCR conditions: Twenty 10-mer oligonucleotide primers (Operon Technology, USA) were randomly chosen for the study. PCR reactions were performed in a total volume of 10 µL reaction mix containing 1 µL of 10× reaction buffer, containing 2 mM $MgCl_2$, 2 µL dNTP at 0.2 mM, 0.1 µL (0.5U) of Taq DNA polymerase (Promega, USA), 30 ng of genomic template DNA and 10 pmol primer in a preheated thermocycler (MJ Research INC, USA). PCR was initiated by a denaturation step at 92°C for 3 min and then the reaction was subjected to 45 cycles of 92°C for 30 sec, 35°C for 1 min, 72°C for 2 min with a final elongation step of 10 min at 72°C. In order to select the optimal conditions of the RAPD-PCR, different optimization experiments were carried out.

Visualization and analysis of RAPD-PCR products: The amplification products were resolved by electrophoresis on a 1.5% agarose gel with ethidium bromide and visualized under UV. The presence and absence of bands between samples was scored and data were transcribed into binary format (1, 0, respectively). Based on the matrix of genetic similarity, cluster analysis was performed. The UPGMA method (unweighted pair-group method with arithmetic averages) was used for clustering employing the NTSYS-pc program [31].

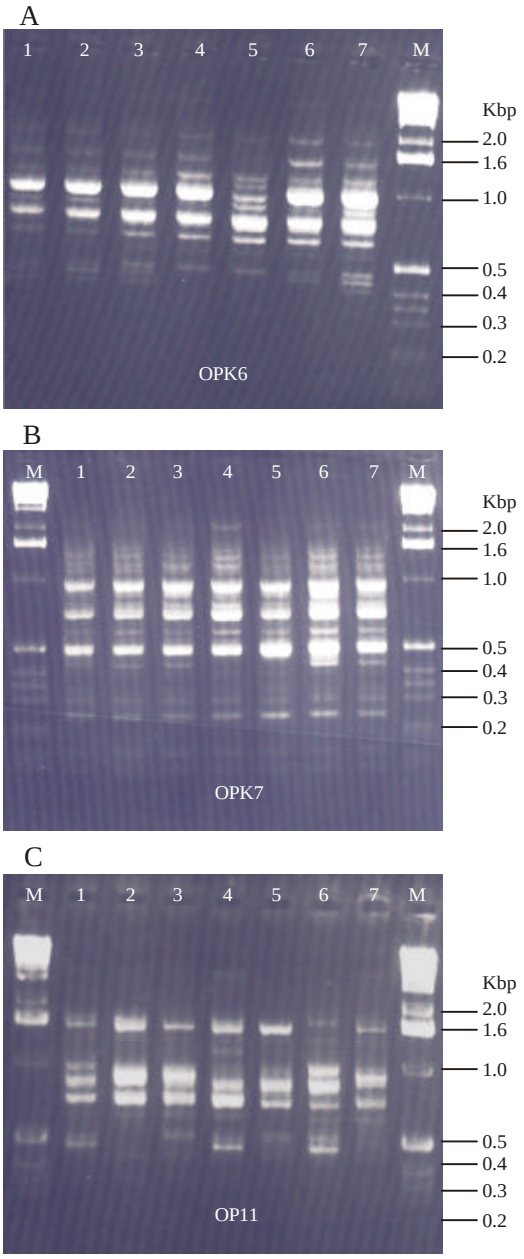

Fig. 1: Agarose gel showing random amplified polymorphic DNA amplification profiles of cultivars analyzed obtained with primer OPK6 (A), OPK7 (B) and OP11 (C). M: 1 kb markers; 1: Camarosa; 2: Chandler; 3: Osogrande; 4: Kabetula; 5: Selected; 6: Rosalinda; 7: Laguna.

RESULTS

RAPD and genetic stability analysis: On the basis of the number, intensity and reproducibility of RAPD bands four primers (OPK7, OPK6, OP3 and OP11) were selected out of the twenty primers, which were previously tested (Data not shown). Bands with the same mobility were treated as identical fragments. Weak bands with negligible intensity and smear bands were both excluded from final analysis. Figure 1 demonstrates the RAPD profiles obtained with three different primers (OPK7, OPK6 and OP11). The number of scored bands varied from eight to thirteen, with an average of 11 bands per primer and an average of 4.5 polymorphic bands per primer. In our analysis of the seven cultivars, the total number of bands scored was 44; with a size range from 200 to 2800 bp. Eighteen bands were polymorphic which represent 40% average RAPD polymorphism. One unique band (approximately 1 kb (Fig. 1C)) was found in each of the cultivars Camarosa and Rosalinda, while was absence of the rest of the seven cultivars analyzed.

Five tissue culture-derived strawberry plants, cv. Rosalinda, showed variations at morphological level. The phenotypic polymorphism was clearly observed in the leaf morphology of the regenerated strawberry plants (Fig. 2A and B). Compared with the control plant (mother plant 'Rosalinda,), there were little differences in the terminal leaflet shape, teeth shape of the leaflet and number of the leaflets. One clone (plant number five) showed simple leaf blade where only one leaflet was seen (Fig. 2A and B). The leaves of the remainder plants were similar to that of the control plant in having three leaflets. The leaflets were found to be pear-shaped with crenate leaflet teeth in case of the tissue culture-derived plants. Whereas leaflets of the control plant were ovate with dentate teeth (Fig. 2A and B).

In order to confirm the genetic stability (at molecular level) of the *in vitro* vegetatively propagated (tissue culture-derived) strawberry clones, the quality of the five tissue cultured-derived regenerants was screened with the twenty random RAPD primers. The DNA was isolated from the leaves of the five tissue culture-derived strawberry plants (produced using the 'Rosalinda' cultivar as mother plant). After RAPD amplification, it was obvious that the five plants (tissue culture-derived) showed identical RAPD profiles (i.e. no polymorphism was observed). As an example, Fig. 2C presented the identical banding patterns of the RAPD profiles (9 bands are observed in case of strawberry clone and their mother parent control) of the five tissue-derived strawberry plants which were amplified using 10-mer OP3 random primer.

Cluster analysis: Genetic similarity among varieties was estimated using dissimilarity coefficient matrix based on RAPD bands scored. Pairwise values of dissimilarity

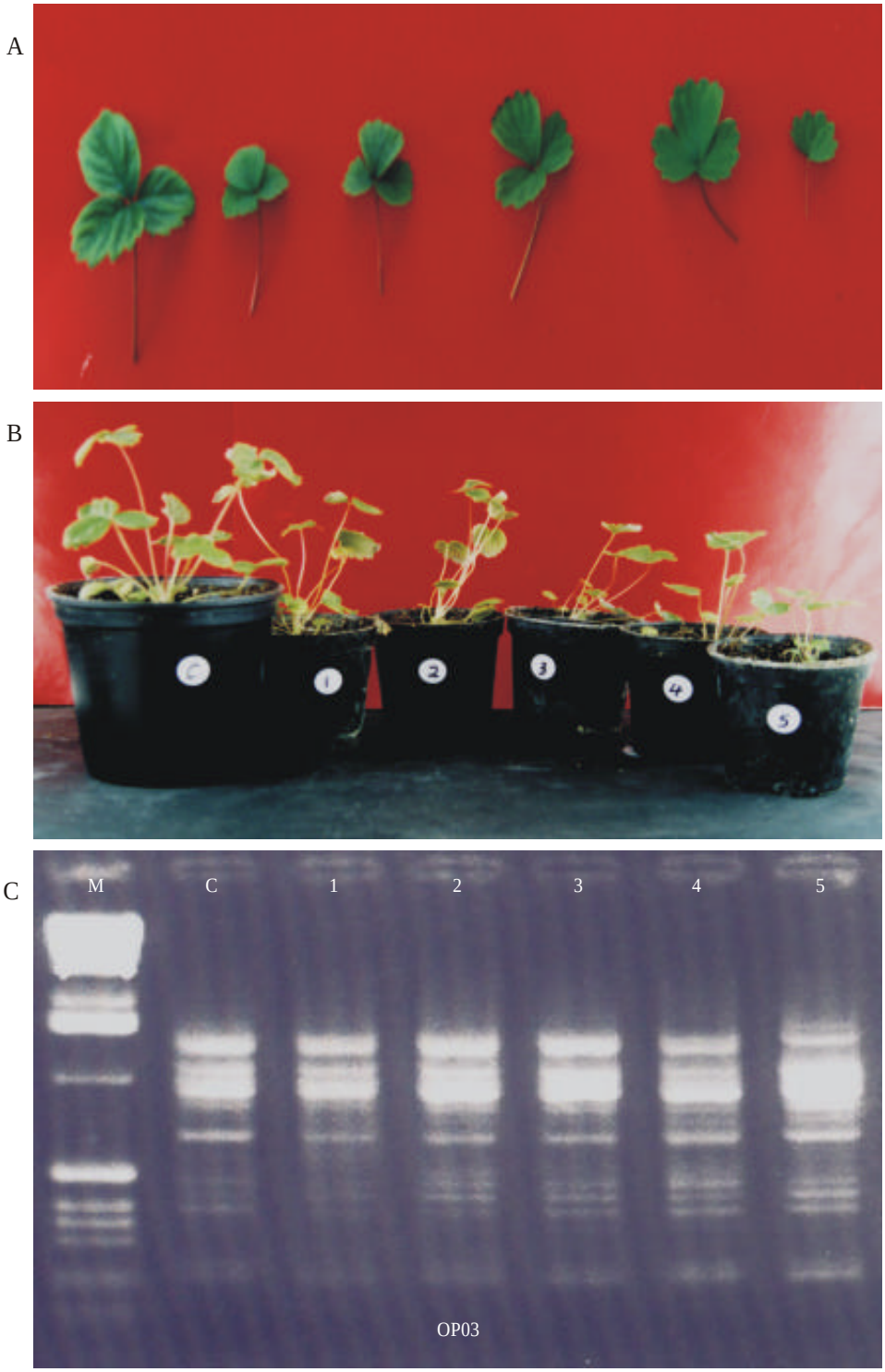

Fig. 2: Morphology of the leaf (A & B) and agarose gel showing random amplified polymorphic DNA amplification profiles of the five tissue culture-derived plants of Rosalinda cultivar (C) obtained with primer OP03.

coefficients ranged from 0.37 for varieties with the same scored bands to 0.56 for the most distant varieties. The dendrogram was constructed based on the dissimilarity matrix, using UPGMA method (Materials and methods). The seven varieties were divided into three different clusters (Fig. 3). The 'Selected' was the most distinct with

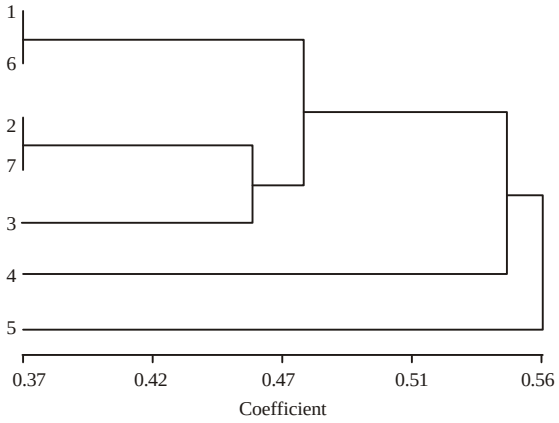

Fig. 3: Dendrogram showing genetic relationships among seven strawberry cultivars based on RAPD data analysis. 1: Camarosa; 2: Chandler; 3: Osogrande; 4: Kabetula; 5: Selected; 6: Rosalinda; 7: Laguna.

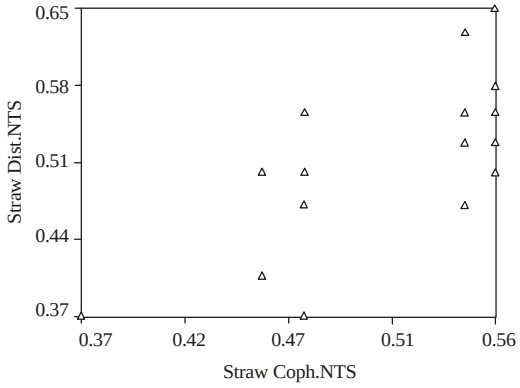

Fig. 4: Mantel t-test comparison of the Dist. and Coph. matrixes of the best dendrogram.

the rest of the cultivars falling under two major groups. The first major group contained only one cultivar the 'Kabetula'. The second major cluster was further separated into three subgroups. The first subgroup contained two varieties Camarosa and Rosalinda, respectively which showed very close RAPD profile (Fig. 1) and exhibited the lowest (0.37) genetic dissimilarity as well. The second subgroup included two cultivars (Chandler and Laguna) which were indistinguishable. While the third cultivar (Osogrande) was highly diverged from them and has represented the third subgroup (Fig. 3). The clusters of the constructed dendrogram, based on the UPGMA, were tested for association. Significant matrix coefficient r-value (0.78) has been obtained which indicated a good fit of the original data for clustering and the obtained phenogram is the most likely one (Fig. 4).

DISCUSSION

Screening of the seven Egyptian strawberry cultivars revealed that banding profiles obtained with OPK7, OPK6, OP3 and OP11 primers were enough to distinguish all the cultivars. The results indicated that the RAPD technique is effective to develop genotype-specific banding patterns valuable for cultivar identification. The obtained results confirm the usefulness and suitability of RAPD markers for strawberry cultivars identification. Our results are in agreement with òebrowska and Tyrka [28], who used RAPD markers to identify and assess the level of genetic diversity among 9 strawberry cultivars differing in their response to photoperiod. They have confirmed the value of RAPD markers for strawberry cultivars identification and author's property rights protection as well as selection of parents suitable for creating of mapping population.

Since RAPD technique does not require previous DNA sequence information and uses very small quantity of DNA, it is considered as one of the most widely used techniques for cultivar identification and genetic diversity studies. However, there is a problem with RAPD regarding its reproducibility. The reproducibility of amplification profiles of RAPD is influenced by any variation in the method used for DNA isolation [32], concentration of template DNA and primer, Taq-DNA polymerase concentration, temperature of annealing, number of thermal cycles and $MgCl_2$ concentration [33, 34]. Several researchers have reported that the majority of RAPD bands are reproducible if one take care in developing a standardized protocol which is strictly followed in each reaction [35, 36]. In order to ensure high RAPD reproducibility, it is essential to optimize the PCR reaction.

RAPD has been used for characterization of different strawberry cultivars [22-24, 26, 28, 37, 38]. In our study, only four RAPD primers (20%) were able to generate polymorphisms among strawberry cultivars. This result is in accord with what have found by òebrowska and Tyrka [28]. Where only three primers were sufficient to identify all materials studied.

In this study, 20 random primers were used in RAPD analysis to prove the clonal fidelity (i.e. genetic stability) of the tissue culture-derived strawberry plants. Identical banding patterns were observed with all the primers tested. These results confirmed the genetic stability of the tissue culture-derived strawberry plants.

Molecular markers are believed to be reliable in monitoring variability at the DNA level in plants. RAPD

technique was used by several research groups to examine genetic variability and it has been found to be very efficient and reliable [39, 40]. As found in the present study, various investigators have observed the absence of variations in *Hordeum* [41], *Picea mariana* [42], *Festuca pratensis* [43], rose [44], Norway spruce [45], *Angelica acutiloba* [46], *Tylophora indica* [40], almond [47] and date palm [16] using RAPD technique. On the contrary, somaclonal variations were found in *Triticum aestivum* [12], *Populus deltoids* [48], sugar beet [39] and peach [49].

In contrast to the RAPD results, minor morphological variation was observed, especially in case of the leaves of tissue culture-derived strawberry plants. The variability observed at the morphological level may be caused by the clonal growth habit of the strawberry [50]. Moreover, clones of many species have a high level of morphological plasticity in response to environmental conditions [51].

These results mean that molecular tools are more reliable than the phenotypic observations for evaluating variations and monitoring genetic stability [52]. It also highlights the need for alternative methods of definitive identification based on molecular techniques such as randomly amplified DNA (RAPDs) or amplified fragment length polymorphism (AFLP).

We demonstrated that RAPD analysis can detect sufficient polymorphism to differentiate among strawberry cultivars and that it is suitable for studying their genetic relationships. This study is considered as a useful report on the assessment of genetic variability of cultivated strawberry genotypes by RAPD molecular markers. Our results showed a much higher level of genetic variability among strawberry cultivars. While no variation was detected among the five tissue culture-derived strawberry plants, which indicated high genetic stability within each cultivar. Therefore, the results of the molecular characterization of Egyptian strawberry cultivars and their genetic relationships provide important parameters for breeding and can be used in the further development of new strawberry cultivars.

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A Comparative Study on the Effect of Foliar Application of Zinc, Potassium and Magnesium on Growth, Yield and Some Chemical Constituents of Mungbean Plants Grown under Water Stress Conditions

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Abstract: Two field experiments were carried out at the Agricultural Experimental Station of National Research Centre, at Shalakan, Kalubia Governorate during the summer seasons of 2002 and 2003 to study the effect of foliar application of zinc, potassium or magnesium on growth, yield and yield components and some chemical constituents of mungbean plants grown under water stress conditions (missing one irrigation at vegetative, flowering and pod formation growth stages). The results revealed that missing one irrigation at any of the three studied stages significantly reduced all the tested growth parameters, yield and yield components as well as photosynthetic pigments content as compared with unstressed plants (control). However, subjecting mungbean plants to moisture stress at vegetative stage had the most negative effect on growth parameters. Meanwhile, stress at a pod formation stage produced the least yield and yield components' values. On the other hand, water stress had a stimulating effect on proline and crude protein contents. The present study also indicate that foliar application of Zn, K or Mg had a positive effect on growth parameters, yield and yield components but K application surpassed the two other nutrients.

Key wards: Mungbean % water stress % zinc % potassium % magnesium %growth % photosynthesis % proline % yield % crude protein % drought susceptibility index

INTRODUCTION

Mungbean (*Vigna radiata* L. Wilczek) is a new introduced summer pulse crop in Egypt with short growing season and high nutritive value grown principally for its protein rich edible seeds [1]. This crop can be used for both seed and forage production. It plays an important role not only in human diet, but also in improving the soil fertility by fixing atmospheric nitrogen into available form with the help of *Rhizobia* species present in the nodules of its roots [2].

Water deficit is frequently the primary limiting factor for crop production under arid and semi-arid conditions [3]. It affects nearly all the plant growth processes. However, the stress response depends upon the intensity, rate and duration of exposure and the stage of crop growth [4].

When considering a watering regime for a crop, it is wise to understand the sensitive growth stages for water stress and the water requirements of the crop in order to achieve maximum yield and maintaining adequate soil moisture conditions during moisture-sensitive stages of growth, so irrigation water may be saved if soil water could be depleted to a greater extent during certain growth stages without affecting yield.

Currently, foliar-applied nutrients have limited direct use for enhancement of stress resistance mechanisms in field crops. Nevertheless, the interactions between plant nutrient levels and stress repair mechanisms are now being studied [5].

Foliar application of potassium during vegetative growth is one of these precautions. Potassium is essential in maintenance of osmotic potential and water uptake and had a positive impact on stomatal closure which increases tolerance to water stress [6]. Moreover, it is involved in activating a wide range of enzyme systems which regulate photosynthesis, water use efficiency and movement, nitrogen uptake and protein building [7]. In this regard, Thalooth *et al.*, [8] found that potassium application improve the water content in the broad bean leaves and the plants showed more tolerance to drought stress.

Another possible approach to minimize drought-induced crop losses is the foliar application of magnesium which plays several physiological and biochemical roles (i.e) chlorophyll formation, activation of enzymes, synthesis of proteins, carbohydrate metabolism and

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energy transfer. Magnesium also acts as a catalyst in many oxidation, reduction reactions inside the plant tissues, as well as it may increase crop resistance to drought. In this concern Saad and El-Kholy [9] stated that foliar application with magnesium sulphate increase net assimilation rates, seed yield and crude protein content of plants.

On the other hand, foliar application of zinc greatly affect plant growth and crop production. In this regard, Krishna [10] reported significant positive effect of zinc treatment on dry matter, seed and straw yield of mungbean as well as crude protein % in the seeds. Kassab [11] indicated that foliar application of Zn, Mg, Mn and Fe significantly increased growth parameters, yield and its components of mungbean plants.

Therefore, this investigation was undertaken to evaluate the efficiency of foliar application of zinc, potassium or magnesium to the harmful effect of water stress on growth and yield of mungbean plants.

MATERIALS AND METHODS

Two field experiments were carried out at the Agricultural Experimental Station of the National Research Centre at Shalakan, kalubia Governorate during the two successive summer seasons of 2002 and 2003 to study the effect of foliar application of Zn, K or Mg on growth, yield and some chemical constituents of mungbean plants grown under water stress conditions. Soil chemical and mechanical characteristic are presented in Table 1.

A split-plot design with four replicates was applied. The main plots included the four irrigation treatments

- Unstressed (control treatment).
- Stressed by skipping one irrigation at vegetative growth stage.
- Stressed by skipping one irrigation at flowering growth stage.
- Stressed by skipping one irrigation at pod formation growth stage.

While the four following foliar application treatments were distributed in the sub plots.

- Foliar application of distilled water (control)
- Foliar application of 300 ppm Zn-EDTA.
- Foliar application of 2.0% KNO_3.
- Foliar application of 50 ppm $MgSO_4$

Foliar application was carried out twice after 30 and 50 days from sowing. Area of each sub plot was 10.5 m^2 (3.5 m length and 3 m width). To avoid the effect of lateral movement of irrigation water, the plots were separated by borders of 1.5 m in width from all sides. Mungbean seeds (*Vigna radiata* L. Wilczek) cv. Kawmy - 1 were sown on 15 and 18 June in the first and second season, respectively in hills 15 cm apart at the two sides of the row.

Table 1: Soil mechanical and chemical analysis (average value of 2002 and 2003 seasons)

Properties	Values
Mechanical analysis	
Clay (%)	33.30
Silt (%)	59.40
Sand (%)	7.30
Soil texture	Clay loam
Chamical analysis	
pH	7.59
Ec (ds mG^{1})	0.27
K^{+} (meq/L)	0.82
Mg^{++} (meq/L)	0.49
Zn^{++} (meq/L)	0.04
HCO_3G (meq/L)	0.41
Ca^{++} (meq/L)	1.09
SO_4G (meq/L)	0.57

Thinning was carried out 21 days after sowing to secure two plants per hill. NPK were added at the rate of 20 kg N/ fed as ammonium nitrate 33% N, 15 kg P_2O_5 /fed as calcium superphosphate 15.5% P_2O_5 (before sowing) and 24 kg K_2O /fed as potassium sulphate 48% K_2O. The other agronomic practice for growing mungbean was followed as recommended. Representative samples were collected from four replicates for each treatment after 75 days from sowing where plant height, leaf area, number of branches, leaves and pods as well as weight of stem, leaves and pods were determined. Proline concentration (µg gG^{1} fresh weight) was determined in the leaves according to the method described by Bates and Tear [12]. Photosynthetic pigments were determined as mg gG^{1} dry weight according to the formula described by Von Wettestein [13].

At harvest time twenty guard plants were chosen randomly from each plot to determine yield attributes ie. number of pods /plant, number of seeds /pod, pods dry weight and seed index. Whole plot was harvested for determination of seed, straw and biological yield /fed. Total nitrogen was determined in the dry seeds using the Kjeldahal method according to A.O.A.C., [14]. Crude protein % was calculated by multiplying the nitrogen % by the factor 6.25.

The Drought Susceptibility Index (S) was calculated for yield data under each stress and foliar application treatment using formula presented by Fischer and Maurer [15].

Table 2: Effect of foliar application with zinc, potassium or magnesium on growth parameter of mungbean plants grown under water stress conditions (Average values of 2002 and 2003 seasons)

Irrigation treatments	Foliar application	Plant height (cm)	Leaf area $(cm)^2$	Number of branches /plant	Number of leaves /plant	Number of pods /plant	Stem dry weight (g)	Leaves dry weight (g)	Pods dry weight (g)
Regular irrigation (control)	Control	67.50	834.35	2.75	16.75	22.25	16.26	14.46	12.40
	Zinc	68.75	888.00	3.00	18.00	25.00	17.60	15.54	13.58
	Potassium	68.25	835.81	3.75	16.75	24.25	16.26	14.46	13.18
	Magnesium	68.50	838.64	3.00	16.50	25.00	16.69	14.63	12.40
Skipping irrigation at vegitative growth stage	Control	55.00	598.37	2.50	12.00	20.25	11.65	10.36	10.59
	Zinc	57.75	608.25	3.00	12.50	21.50	12.33	10.79	11.37
	Potassium	58.25	641.13	2.75	13.25	23.75	12.86	11.44	12.55
	Magnesium	59.75	591.69	2.50	12.00	22.25	11.55	10.39	11.24
Skipping irrigation at flowering growth stage	Control	58.25	610.73	2.75	12.25	20.25	11.89	10.58	10.59
	Zinc	60.00	627.42	3.00	13.25	20.50	13.14	11.44	10.85
	Potassium	58.75	652.03	2.50	13.50	21.00	13.11	11.65	11.24
	Magnesium	59.25	612.99	2.75	12.50	20.50	11.65	10.58	10.81
Skipping irrigation at pod initiation growth stage	Control	63.00	642.91	2.75	13.25	19.50	12.86	11.44	9.95
	Zinc	64.75	653.40	3.00	13.75	20.00	13.71	11.87	10.21
	Potassium	62.50	677.04	2.75	14.50	20.00	14.08	12.52	10.34
	Magnesium	63.25	645.01	2.75	13.50	20.25	12.86	11.54	10.36
Irrigation mean	Control	68.25	849.20	3.13	17.00	24.13	16.70	14.77	12.89
	Vegitative	57.69	609.86	2.69	12.44	21.94	12.10	10.75	11.44
	Flowering	59.06	625.79	2.75	12.88	20.56	12.45	11.06	10.87
	Podding	63.38	654.59	2.81	13.75	19.94	13.38	11.84	10.21
Foliar application mean	Control	60.94	671.59	2.69	13.56	20.56	13.17	11.71	10.88
	Zinc	62.81	694.27	3.00	14.38	21.75	14.20	12.41	11.50
	Potassium	61.94	701.50	2.94	14.50	22.25	14.08	12.52	11.83
	Magnesium	62.69	672.08	2.75	13.63	22.00	13.19	11.79	11.20
LSD 5%	Irrigation	1.88	78.92	0.55	1.59	1.58	1.44	1.33	0.77
	Foliar	NS	40.76	0.49	0.76	1.49	0.74	0.59	0.64
	Irrig x Foliar	4.60	81.53	0.98	1.53	2.97	1.48	1.19	1.29

$$S = (1 - YD /YP) /D$$

Whereas; S = Drought susceptibility index, YD = Yield of plants under drought stress condition, YP = Yield of plants without drought (under normal irrigation treatments), D = Drought intensity = 1 - (mean YD of all treatments /mean YP of all treatments).

The obtained results were subjected to statistical analysis of variance according to the method described by Snedecor and Cochran [16] and the combined analysis of the two seasons was calculated according to the method of Steel and Torrie [17].

RESULTS AND DISCUSSION

Effect on growth: The growth parameters as affected by irrigation treatment and foliar application of the different nutrients are presented in Table 2. However, all growth parameters i.e. plant height, number of branches, leaves and pods /plant, weight of stem, leaves and pods /plant as well as leaf area /plant were significantly reduced by skipping one irrigation at the different stages of growth as compared with the control plants. The magnitudes of reduction differed from character to another according to the growth stage. However, subjecting plants to water stress at vegetative stages of growth caused the highest reduction in all growth parameters except that of number and weight of pods /plant which reduced by stress at pod formation stage of growth. These results were in harmony with those obtained by Thomas *et al.*, [18]. The depressing effect of drought on plant growth was suggested to be attributed to the increase osmotic pressure in the root medium which tend to decrease synthesis of metabolites, reduce translocation of nutrient from the soil to the plant as well as decrease division and elongation of the cells [19]. Moreover, Maiti *et al.*, [20], added that water stress progressively declined net photosynthesis rate which is associated with a simultaneous decrease in leaf area, decrease starch content and increased free proline.

Regardless irrigation treatments, results in Table 2 reveal also that foliar application of Zn or K significantly increased all growth parameters. Differences among the effect of the foliar application were recorded. K was superior in the features of area, number and weight of leaves /plant as well as number and weight of pods /plant, while Zn surpass the features of plant height, number of branches /plant and stem dry weight /plant. On the other hand, application of Mg insignificantly increases all growth parameters as compared with control plants. These results coinside with those obtained by Basole *et al.*, [21], Gupta *et al.*, [22] and Kassab [11].

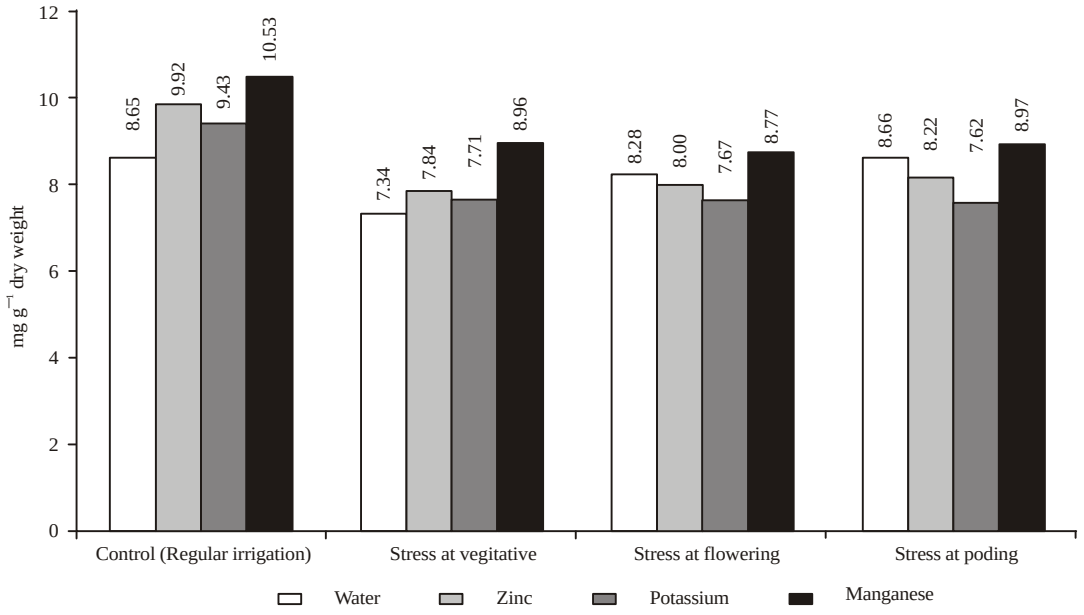

Fig. 1: Effect of foliar application of Zn, K and Mg on chl. a+b (mg gG^{1} dry weight) in the leaves of mungbean plants grown under water stress conditions. (LSD 5% NS)

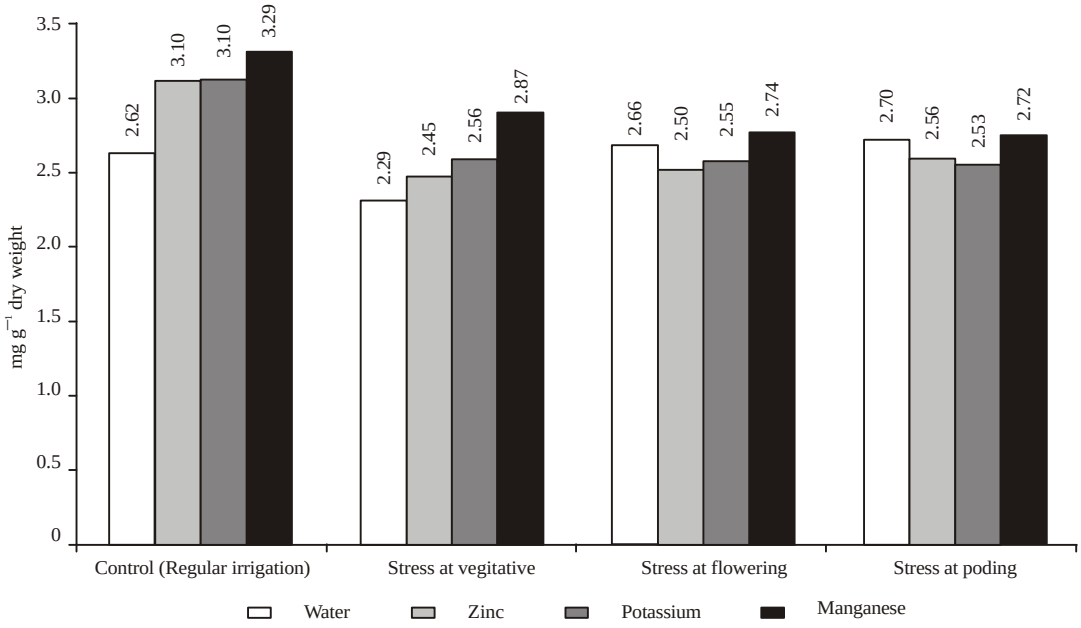

Fig. 2: Effect of foliar application of Zn, K and Mg on carotenoids content (mg gG^{1} dry weight) in the leaves of mungbean plants grown under water stress conditions. (LSD 5% NS)

It can be concluded also that the enhancement effect of spraying mungbean plants with Zn, K or Mg on growth parameters was very clear, hence treated plants resulted in taller, greater number and weight of leaves, branches, pods /plant. Such enhancement effect might be attributed to the favorable influence of these nutrient on metabolism and biological activity and its stimulating effect on photosynthetic pigments and enzyme activity which in turn encourage vegetative growth of plants [23].

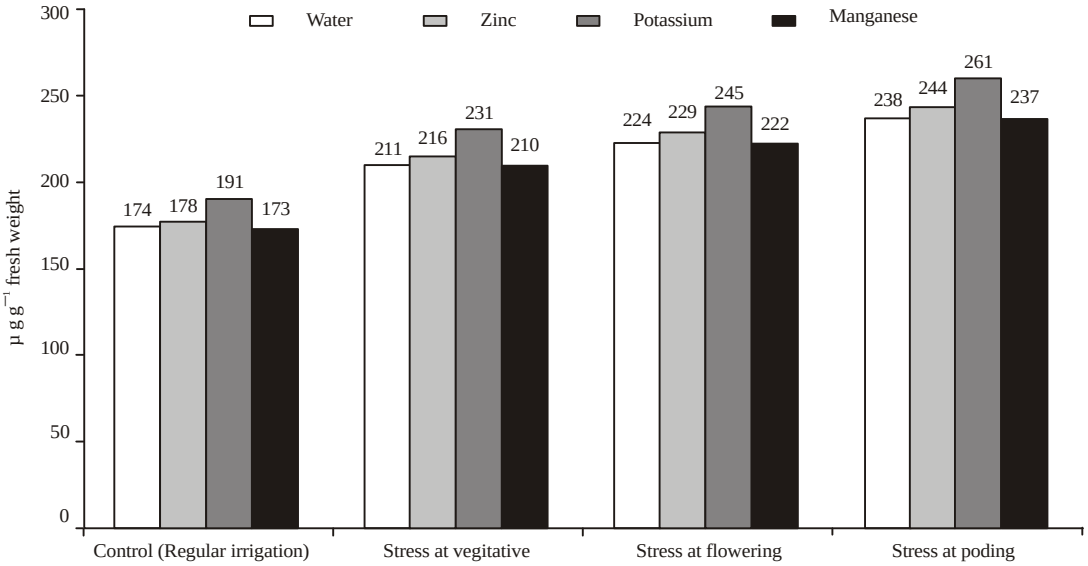

Fig. 3: Effect of foliar application of Zn, K and Mn on proline content µg g^{-1} fresh weight in the leaves of mungbean plants grown under water stress conditions. (LSD 5% 14.89)

The interaction between irrigation treatment and foliar application of the different nutrients significantly affected all the studied growth parameters. However, foliar application of Zn or K recorded the highest values for all growth parameters under normal irrigation treatment (control). On the other hand, skipping one irrigation at vegetative stage of growth x foliar application of distilled water treatment gave the lowest values for most growth parameters. Similar results were obtained by Thomas *et al*., [18]. Such differences might be due to the variation existing among the role of plant nutrients on stress resistance and repair mechanisms [23].

Effect on photosynthetic pigments content: Data presented in Figs. 1 and 2 revealed that withholding one irrigation at any growth stage decreased the content of chl. a+b and carotenoids in the leaves of mungbean plants and this was true under the different foliar nutrients application. Similar results were obtained by Maiti *et al*., [20]. Such decrease in chlorophyll content in the leaves of plants may be attributed to the high rate of chlorophyll degradation more than its biosynthesis under water stress conditions [24]. Furthermore, Schtz and Fangmeier [25], added that, water stress accelerate chlorophyll-a breakdown.

The same Figures also show that there was insignificant increase in photosynthetic pigments content (chl. a+b and carotenoids) under foliar application with Mg as compared with the other treatments. Such enhancing effect of Mg on chlorophyll accumulation could be attributed to the useful importance of magnesium for photosynthesis, net assimilation and transpiration rates [26]. On the other hand, the negative effect of foliar application of K on chlorophyll content may be attributed to the antagonism between K and Mg. In this respect Kabesh *et al*., [27], observed that excess of K supply depress the uptake of Mg and this can induce Mg deficiency. The reduction in Mg content in plant leaves often causes inhibition of chlorophyll biosynthesis, since Mg is one of the main constituents of chlorophyll molecule. However, no clear effect was observed for zinc application. The previous results are in agreement with those obtained by Nobel [26].

Regarding the interaction effect between water stress and different types of foliar application on photosynthetic pigments content, the highest photosynthetic pigments was recorded in mungbean plants under regular irrigation treatment and foliar application of magnesium, while the least values was obtained by plants stressed at vegetative and treated with potassium. These results are in full agreement with those obtained by Kabesh *et al*., [27] and Maiti *et al*., [20].

Effect on proline content: From the data given in Fig. 3, it can be concluded that skipping irrigation at any growth stage significantly increased proline content in the leaves of mungbean plants as compared with the unstressed plants (control). It is worthy to note also that high proline content values were recorded in mungbean plants subjected to water stress at pod formation stage. The obtained results are in agreement with the findings of

Table 3: Effect of foliar application with zinc,potassium or magnesium on yield of mungbean plants grown under water stress conditions (Average values of 2002 and 2003 seasons)

Irrigation treatment	Foliar application	Number of pods /plant	Pods dry weight (g)	Number of Seeds /pod	Seed dry wt. g /plant	Seed index	Seed yield kg /fed.	Straw yield kg /fed.	Biological yield kg /fed.
Regular	Control	26.73	18.74	9.25	10.72	55.63	730.16	2199.67	2929.84
irrigation	Zinc	30.03	21.06	9.25	11.99	62.50	816.77	2535.75	3352.52
(control)	Potassium	29.79	20.89	9.50	11.84	60.63	806.25	2378.98	3185.23
	Magnesium	30.03	21.06	9.50	11.94	62.50	813.32	2450.18	3263.50
Skipping	Control	23.87	16.74	9.00	8.89	50.63	605.86	1648.46	2254.32
irrigation	Zinc	25.35	17.77	9.50	9.44	53.75	643.26	1723.34	2366.60
at vegitative	Potassium	28.40	19.92	9.25	10.71	59.38	729.26	1632.71	2361.98
growth stage	Magnesium	26.23	18.39	9.25	9.77	55.63	665.70	1711.86	2377.56
Skipping	Control	23.43	16.43	8.50	8.73	50.63	594.56	1791.15	2385.70
irrigation	Zinc	23.72	16.63	9.00	8.84	51.25	601.90	1888.42	2490.32
at flowering	Potassium	24.59	17.24	8.75	9.29	52.50	632.57	1780.58	2413.16
growth stage	Magnesium	23.72	16.63	8.50	8.84	51.25	601.90	1813.26	2415.16
Skipping	Control	21.56	15.12	8.25	8.03	48.75	547.19	1825.21	2372.40
irrigation at	Zinc	22.11	15.51	8.00	8.24	50.00	561.22	1991.76	2552.99
pod initiation	Potassium	22.33	15.66	8.50	8.44	50.00	575.14	2046.98	2578.57
growth stage	Magnesium	22.39	15.70	8.25	8.34	50.63	568.24	2005.48	2573.71
Irrigation	Control	29.14	20.44	9.38	11.62	60.31	791.62	2391.15	3182.77
mean	Vegitative	25.96	18.21	9.25	9.70	54.84	661.02	1679.09	2340.12
	Flowering	23.86	16.73	8.69	8.92	51.41	607.73	1818.35	2426.08
	Podding	22.10	15.50	8.25	8.26	49.84	562.95	1967.36	2519.42
Foliar	Control	23.90	16.76	8.75	9.09	51.41	619.44	1866.12	2485.57
application	Zinc	25.30	17.74	8.94	9.63	54.38	655.79	2034.82	2690.61
mean	Potassium	26.28	18.43	9.00	10.07	55.63	685.81	1959.81	2634.73
	Magnesium	25.59	17.95	8.88	9.72	55.00	662.29	1995.20	2657.48
LSD 5%	Irrigation	1.88	1.32	0.75	0.80	3.96	54.52	157.48	210.83
	Foliar	1.61	1.13	0.49	0.60	3.72	40.72	101.38	130.03
	Irrig x Foliar	3.22	2.26	0.98	1.20	7.44	81.43	202.76	260.06

Maiti *et al*., [20], who reported that proline accumulation is a mechanism for plants adaptation to abiotic stress conditions. Other roles for proline have been proposed, including stabilization of macromolecules, a sink for excess reductant and a store of carbon and nitrogen for use after relief of water deficit [28].

Figure 3 showed also that, foliar application of potassium significantly increased proline content, while no clear effect was observed for zinc or magnesium. Similar results were obtained by Nobel [26].

Regarding the effect of interaction between water stress and foliar treatments, data presented in Fig. 3 reveal also that the highest value of proline content (261 µg gG^{1} fresh weight) was recorded by mungbean plants stressed at pod formation and treated with foliar potassium, whereas the lowest value (173 µg gG^{1} fresh weight) was obtained by plant irrigated regularly (control) and treated with foliar application of magnesium. Basole *et al*., [21] came to the same conclusion. In this connection, Yang *et al*., [24], found that highly drought resistance plants reduce their water loss by increasing proline content.

Effect on yield and yield components: The data presented in Table 3 show that skipping one irrigation at any stage of growth significantly decreased yield and yield components of mungbean plants. However subjecting plants to water stress at pod formation stages of growth caused the highest reduction in number of pods /plant, pods dry weight, number of seeds /pod, seeds dry weight /plant, seed index and seed yield kg /fed. Stress at flowering came in the second order with respect to these features, while early stress at vegetative has more detrimental effect on straw and biological yield. These results are in full agreement with those obtained by Thomas *et al*., [18]. The expected depression as a result of water stress on yield and yield components of mungbean plants may be due to the reduction of growth criteria as indicated in Table 2. In this concern, De Costa *et al*., [29] stated that irrigation is critical during pod-filling and flowering stages in mungbean plants mainly because of the higher leaf area index during these periods and consequently, the greater demand for water. In addition, Dominique *et al.*, [30] stated that, early stress reduced vegetative biomass production and decreased the average internodes length without altering the efficiency of dry matter for producing and filling pods and seeds. They added that decreased pods weight was an effective indicator of water limitation during pod lengthening. It is clear also from the same table that mungbean plants appeared to be more sensitive to water stress during pod formation stage. Moreover, Kramer [31] reported that

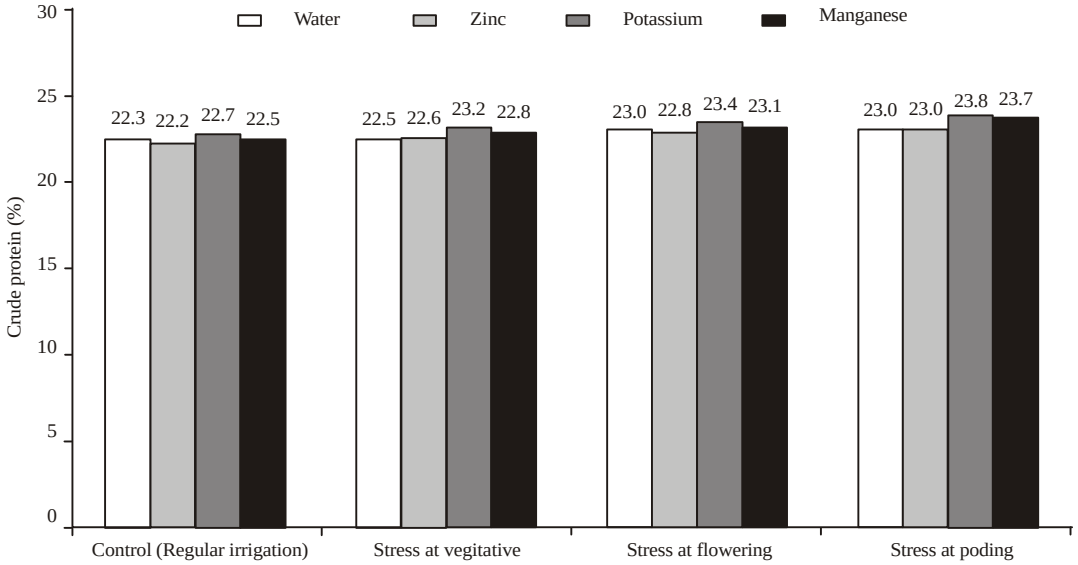

Fig. 4: Effect of foliar application of Zn, K and Mn on crude protein % in the seeds of mungbean plants grown under water stress conditions. (LSD 5% 0.89)

water stress resulted in a disturbance in photosynthesis, enzyme activity and protein synthesis which affect the metabolites transportation to the grains.

Irrespective to water stress, foliar application of Zn, K, or Mg significantly increased all the yield characters compared with control plants. Potassium foliar application had the greatest stimulatory effect on pods number /plant, pods dry weight, number of seeds /pod seeds dry weight /plant, seed index and seed yield kg /fed. On the other hand, zinc application was superior with respect to straw and biological yield /fed. The obtained result are in full agreement with the findings of Basole *et al.,* [21], Gupta *et al.,* [22] and Kassab [11]. These results suggested that foliar application of nutrient solutions partially alleviates the adverse effects of water stress on photosynthesis and photosynthesis-related parameters, yield and yield components through mitigating the nutrient demands of water-stressed plants. In this concern, Ved *et al*., [32] stated that foliar applied zinc enhances photosynthesis, early growth of plants, improves nitrogen fixation, grain protein and yields.

As for the interaction effect of water stress and foliar application treatment. Table 3 reveal that the highest values were recorded by the plants irrigated regularly (control) and sprayed with potassium. These results were parallel with those obtained by Basole *et al.,* [21] and Gupta *et al.,* [22], who observed a significant increase in yield under water stress condition in response to foliar K application. On the other hand, the lowest values were recorded by the plants stressed at pod formation and treated with distilled water. Similar results were obtained by Gupta *et al.,* [22] and Kassab [11].

Effect on crude protein % in the seeds: Data presented in Fig. 4 show that, subjecting mungbean plants to moisture stress at different stages of growth significantly increased the seed crude protein content. However, skipping one irrigation at pod formation stages recorded the highest crude protein % compared with the other treatments. These results are in agreement with those obtained by Kassab [11]. In this concern, Zhao *et al.,* [33] stated that water stress at grain filling stage can increase grain protein content.

Regardless irrigation treatment, foliar application of potassium significantly surpassed the other treatments with respect to crude protein percentage. Magnesium came in the second order, while no clear effect for zinc was observed. Similar results were obtained by Kassab [11] Such enhancement effect of potassium and magnesium might be attributed to the favorable influence of these nutrient on metabolism and biological activity and its stimulating effect on photosynthetic pigments and enzyme activity which in turn encourage vegetative growth and yield of plants and consequently protein content [23].

The interaction between moisture stress and foliar application significantly affect crude protein percentage. The highest value (23.8%) was recorded in plants stressed at pod formation and treated with potassium, while the

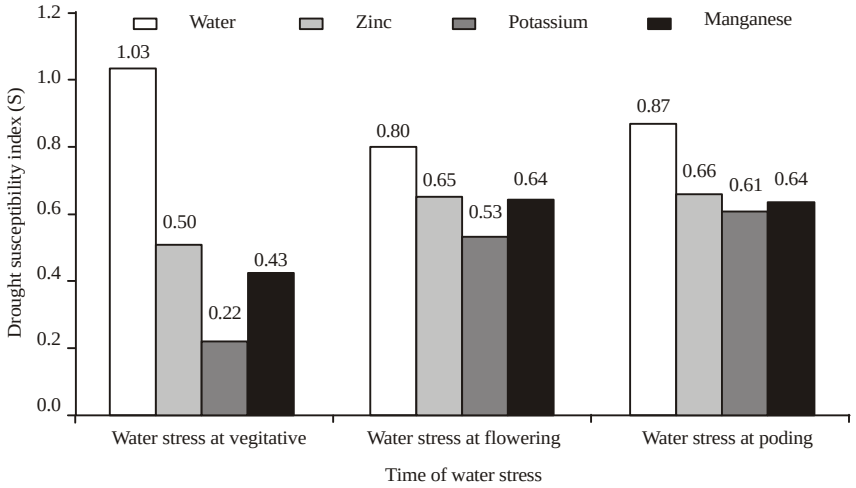

Fig. 5: Drought susceptibility index of mungbean plants as affected by moisture stress and some foliar nutrients application

least one (22.2) was recorded in unstressed plants and sprayed with Zn. Similar results were obtained by Gupta *et al.,* [22].

Effect on drought Susceptibility Index (S): Data presented in Fig. 5 show Drought Susceptibility Index (S) under each drought stress treatment. It is evident that subjecting mungbean plant to water stress at pod formation stage of growth has the most deleterious effect on its productivity. In other wards it means that mungbean plants are very susceptible to drought stress at this development stage of growth. On the other hand it can be noticed that mungbean plants are more tolerant to drought stress during vegetative stage of growth. Similar results were obtained by Tawfiles [34].

However, foliar application of Zn, K or Mg increased drought tolerance of mungbean plants according to Ceccarelli [35], who reported that low (S) value is an indication to drought tolerance.

It can be concluded that treatment mungbean plants grown under water stress conditions with K or Mg is solutions counteracted the deleterious effects of stress on the yield, especially the stress at early stages of growth and helped stressed plants to grow successfully under these adverse unfavorable conditions. Moreover, applying Zn is very effectively in case of using mungbean plants as forage. However this subject needs more and deeper study for achieving clearer conclusion.

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Stability Parameters in Yield of White Mustard (*Brassica Alba* L.) in Different Environments

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Abstract: Stability parameters of 20 genotypes of white mustard were evaluated under four environments in two locations and assessed using three different stability methods. The investigation included five characters (plant height, number of primary branches, number of secondary branches, number of pods on main branch and seed yield). Results revealed significant genotype × environment interactions for all studied traits and the response to environmental changes of each genotype differed as indicated by M.S. pooled deviation and heterogeneity items. Wider ranges of regression coefficient values were observed from the studied stability methods suggesting possibility of selection for specific genotypes patterns. Four genotypes (No. 6, 10, 12 and 13) were most stable for studied characters in four environments. Genetic characterization of white mustard genotypes by SDS-PAGE analysis of protein fractions revealed differences in the banding profile pattern in the altered environment (clay vs. sandy soils). Moreover, some other protein bands were also found in the sandy soil more than in the clay soil.

Key words: Selection % white mustard % genotype-environment interaction % stability measurements

INTRODUCTION

Stability of production under different environments is an important consideration in medicinal plants breeding programs. Some genotypes may fair well in some environments but no so well in others [1]. The development of varieties, which adapted to a wide range of diversified environments, is ultimate goal of plant breeders in crop improvement programs. The adaptability of a variety over diverse environments is usually tested by the degree of its interaction with different environments under which it is planted. A variety or genotype is considered to be the most adaptive or stable one if it has a high mean yield but a low degree of fluctuation in yielding ability when grown over diverse environments [2]. Many investigators among them [3-7], described the importance of genotypes x environmental interaction in stability analysis. White mustard (*Brassica alba*, L.) is an erect annual crop, cultivated as oilseed crops and adapted to wide variety of climatic conditions and suited to many types of soils [8]. It was also used in herbal medicine as antibacterial, antifungal carminative, diuretic, Emetic, Expectorant, Stimulant and ruberfacient [8-11].

Some methods have been proposed to evaluate stability [12-14]. They divided the variance due to environment into combined regression and environmental residual. They also divided the variance due to a genotype x environment interaction into heterogeneity of regression and residual.

The present investigation was an attempt to study the stability of some white mustard genotypes yield and yield components characters under different environmental conditions (clay and sandy soils). In addition, the pattern of proteins electrophoresis of different environments were characterized by gel filtration and SDS-Polyacralymide gel electrophoresis.

MATERIALS AND METHODS

Seed material used in this study was 20 genotypes of white mustard (*Brassica alba*, L.) which were sown at the Experimental Farm Station of National Research Center (NRC) at Shalakan, Kalubia Governorate (clay soil) and at Farm of South El-Tahrir Agricultural Company, El-Behira Governorate (sandy soil), during two successive growing seasons (2002/ 2003) and (2003/ 2004).

Sowing was done in a randomized complete blocks design with three replications in each above mentioned environments. Planting dates were at 22nd October 2002 and 28th October 2003, respectively. At full ripen,

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five plants of each replicate per each entry of different generations were harvested and the plant records were considered as already mentioned. Data recorded on:

- Plant height (cm)
- Number of primary branches
- Number of secondary branches
- Number of pods on main branch
- Seed yield per plant (g)

Statistical analysis: A combined analysis of variance was used to evaluate the responses of each character within the experiment and to determine the genotype-environment interaction. Whenever, the variance due to genotype-environment interaction was significant, the analysis was continued in order to estimate the stability parameters. Stability analysis was computed according to Eberhart and Russell [12]. To detect the phenotypic stability under different environments:

$$Y_{ij} = \mu_i + \$_i\ I_j + *_{ij}$$

Where; Y_{ij} = Genotype mean of i^{th} genotypes at j^{th} environments, μ_i = Mean of all genotypes over all environments, $\$_i$ = the regression coefficient of the i^{th} genotypes on the environmental index, which measure the response of this genotype to varying environments, I_j = Environmental index, which is defined as the deviation of the mean of all genotypes at a given environment from the grand mean, and $*_{ij}$ = the deviation from regression of i^{th} genotypes at j^{h} environments.

Perkins and Jinks [13], proposed a different model for stability analysis. In this model, the total variance is first divided into three components, viz. (1) genotypes (G), (2) environments (E) and (3) genotypes x environment. The G x E variance is subdivided into (a) heterogeneity due to regression and (b) sum of square (SS) due to remainder. The S.S remainder is further divided into S.S due to individual genotype. The main features of this model includes three parameters of stability like [12], with one exception; the degree of freedom for environment is e-2. Another objection of [14], to other models was about the partitioning of the degree of freedom. Though, S.S. due to environment (linear) of [12], being the same as S.S. due to environment (joint regression) of Perkins and Jinks model, yet the degree of freedom is one in the former and s-1 in the latter. In Eberhart and Russell model, b (regression coefficient) is considered as parameter of response and S^2_d as the parameter of stability. As far as the ranking of genotypes with respect to there stability is considered, it remains the same under all the three models described above. Eberhart and Russell's model bing relatively simple, may, therefore, be preferred for studying stability analysis.

The model of Perkins and Jinks [13].

$$Y_{ijk} = \mu + a_i + g_j + r_{jk} + \$_{-i} g_j + *_{ij} + e_{ijk}$$

Where; Yijk: is the mean performance of the line i in replicate k of environment j, μ is the overall mean, a_i is the contribution of line i, g_j is the contribution of environment j, r_{jk} is the contribution of replicate k in environment j, $\$_{-i}$ is the linear regression coeffecient for line i, $*_{ij}$ is the deviation from regression, and e_{ijk} is the residual variation of line i in replicate k in of environment j.

Freeman and Perkins [14], proposed independent estimate of environmental index in the following two ways:

1) Divide the replications into groups, so that the one group may be used for measuring the average performance of genotypes in various environment and the other group, averaging over the genotypes is used for estimating the environmental index.
2) Use one or more genotypes as check and assess the evironmental index on the basis of there performance.

The hypothesis that any regression coefficient does not differ from unity was tested by the T-test [15], using its own standard error for regression. Also the mean square of deviation from regression of each genotype (S^2_d), pooled errors in the regression analysis of variance were used to test whether each deviation mean square was significantly different from zero.

Wricke and Weber [16], proposed ecovalence model to evaluate the balanced response of G x E interaction as follows:

$$W_i = 3_j (Y_{ij} - Y_{i.} - Y_{.j} + Y..)^2$$

Where: W_i is the ecovalence of the i^{th} genotypes, Y_{ij} is the mean performances of genotype (i) in the j^{th} environment, $Y_{i.}$ and $Y_{.j}$ are the genotype and environment mean deviations, respectively and Y.. is the over all mean.

Oil content (%): The oil was extracted on basis of air-dried seed from a random sample of each types of entries. Soxhelt extraction method was used to determine oil content by hexane solvent which described by AOAC [17].

Gel electrophoresis: Total proteins electrophoresis analysis were carried out according to Laemmli [18]. Seeds of four entries of genotypes were defatted with hexane for one week and ground in liquid nitrogen. One milliliter of water soluble extraction buffer was added. After centrifugation for 10 min, a 12,000 rpm under 4°C, the supernatant was collected [19]. Electrophoresis was carried out at 4°C until the bromophenol blue front passed completely through the gel. The gel was stained for 12 h in 0.1% coomassie brilliant blue and distained until the bands were clearly observed.

RESULTS AND DISCUSSION

Data presented in Table 1 indicated that significant differences among genotypes, environments and genotype × environment interaction were detected for all studied traits. These results revealed that mustard genotypes responded differently to the different environmental conditions. This finding suggested the importance of assessment of genotypes under different environments to identify the best genetic makeup for a particular environment. These findings were agreement line with those previously obtained by Ali *et al*., [6], Wani [20] and Ali *et al*., [21].

The differences between grand mean (over all environments) and each of the four environmental mean performances for the five studied traits recorded covered a wide range and displayed a good distribution within the range as shown in Table 2. Consequently, the required assumptions for stability analysis is full-filled [22]. Number of secondary branches differences ranged from 2.90 in the second environment to 2.67 in the first environment.

Eberhart and Russell [12], model provides a mean of partitioning the genotype-environment interaction for each genotype into two parts.

- The variation due to the response of genotype to varying environmental index (sum of squares due to regression).
- The unexplainable deviation from the regression on the environmental index. They added that a stable genotype could have high mean performance.

Significant genotypes × environments (Linear) interaction were detected for all studied traits Table 3. This result indicated that the differences among genotypes for their regression on the environmental index proceeded further to estimate the (bi) values. Pooled deviations mean squares were insignificant suggesting linear regression also assume partial importance considering each individual genotype

The joint regression analysis was conducted for all studied traits according to the procedure described by Perkins and Jinks [13]. All sources of variation mean squares were tested against average error Table 4.

Highly significant differences among genotypes and environments were found for all studied traits. Also, there were high significant differences among genotype x environment interaction for all studied traits. On the other side, heterogeneity between regression mean squares were highly significant when tested against the remainder mean squares for plant height, number of primary branches, number of pods/ main branch and seed yield/ plant. However, the remainder mean squares were highly significant for all traits except number of secondary branches when tested against average error.

Table 1: The combined analysis of variance of all studied traits for 20 white mustard genotypes over four environments tested

S.O.V.	d.f.	Plant height	No. of primary branches	No. of secondary branches	No. of pods/ main branch	Seed yield/ plant
Environments (E)	3	7301.75**	751.84**	7.14**	2137.61**	1606.82**
Rep./ Env.	8	53.63	3.67	0.78	15.04	19.91
Genotypes (G)	19	1379.65**	33.07*	4.38**	1280.54**	641.28**
G x E	57	567.64**	15.29**	0.91**	45.54**	44.87**
Error	152	27.02	2.47	0.38	9.80	8.98

*, ** Denote significant at 0.05 and 0.01 probability levels, respectively

Table 2: Mean performance of all traits studied under each of the four environments tested

Environments		Plant hieght	No. of primary branches	No. of secondary branches	No. of pods/ main branch	Seed yield/ plant
1		121.67	20.32	3.67	32.47	27.62
2		106.78	9.42	2.90	24.05	25.83
3		133.48	15.92	3.25	37.27	37.55
4		123.72	9.17	2.98	26.45	29.42
Average		121.41	13.70	3.20	30.06	30.11
LSD	0.05	3.09	0.81	0.37	1.64	1.88
	0.01	4.49	1.18	0.54	2.38	2.74

Table 3: Pooled analysis of variance for all studied traits for the 20 white mustard genotypes under two locations over two years, Eberhart & Russell [12]

S.O.V.	d.f.	Plant height	No. of primary branches	No. of secondary branches	No. of pods/ main branch	Seed yield/ plant
Genotypes (G)	19	459.89**	11.03**	1.46**	42.85**	213.76**
Environments (E)+G x E	60	339.56	28.50**	0.66**	48.32**	82.54**
G x E (linear)	19	226.61*	9.93**	0.65**	22.69*	22.34*
Pooled deviation	40	161.99	2.54	0.12	10.85	10.70
1	2	197.64**	0.54	0.41**	22.16**	30.61**
2	2	139.23**	6.28**	0.14	11.65**	51.64**
3	2	1017.64**	7.77**	0.03	11.44**	7.46*
4	2	165.11**	1.41	0.03	11.51**	2.47
5	2	267.07**	2.18**	0.27*	24.09**	26.00**
6	2	20.94*	1.05	0.33**	3.11	3.40
7	2	72.74**	6.96**	0.05	0.60	0.28
8	2	154.28**	1.22	0.02	4.85	4.16
9	2	210.08**	1.41	0.10	20.74**	16.35**
10	2	26.92**	2.32**	0.18	5.62	17.62**
11	2	60.68**	0.76	0.38**	3.84	8.53**
12	2	46.54**	4.36**	0.13	4.32	4.81
13	2	53.27**	0.69	0.01	5.11	0.37
14	2	115.50**	0.51	0.01	2.89	4.32
15	2	11.16	0.76	0.05	31.15**	5.77
16	2	108.94**	3.29**	0.02	7.25*	8.31**
17	2	320.44**	1.98*	0.03	14.39**	9.49**
18	2	172.32**	1.96*	0.12	13.95**	6.47*
19	2	8.18	0.85	0.09	11.03**	0.02
20	2	71.21**	4.58**	0.05	7.38*	5.97*
Pooled error	160	9.005	0.82	0.13	3.27	2.99

*, ** Denote significant at 0.05 and 0.01 probability levels, respectively

Table 4: The joint regression analysis of variance for all studied traits over two locations and two growing seasons (Pirking and Jinks model [13])

S.O.V.	d.f.	Plant height	No. of primary branches	No. of secondary branches	No. of pods/ main branch	Seed yield/ plant
Genotypes (G)	19	459.89**	11.03**	1.46**	42.85**	213.76**
Environments (E) (joint regression)	3	2433.98**	583.95**	2.38**	712.53**	535.61**
G x E	57	189.22**	5.10**	0.30**	15.18**	14.96**
Heterogeneity between regression	19	226.6**	9.93**	0.65**	22.69**	22.34**
Remainder	38	170.52**	2.68**	0.13	11.43**	11.27**
Pooled error	160	8.55	0.78	0.12	3.10	2.84

** Denote significant at 0.01 probability level

Table 5: Partitioning of analysis of variance for all studied traits over two locations and two growing seasons, according to freeman and Perkins [14]

S.O.V.	d.f.	Plant height	No. of primary branches	No. of secondary branches	No. of pods/ main branch	Seed yield/ plant
Genotypes (G)	19	954.84	22.97*	3.30	93.17	412.17*
Environments (E)	3	4911.58*	1132.66**	7.00	1374.85*	977.95*
Combined regression	1	14364.92	3397.06	17.79	3988.05	2889.52
Residual regression	2	184.92	0.46	1.61	68.25	22.16
G x E	57	384.78	11.53	0.74	29.65	32.40
Heterogeneity of rgression	19	478.84	24.77**	1.21**	41.40*	47.38*
Residual	38	337.74	4.91	0.51	23.78	24.91
Error between replicates	80	703.38	58.29	1.97	104.47	166.33

*, ** Denote significant at 0.05 and 0.01 probability levels, respectively

The partitioning analysis of variance model of Freeman and Perkins [14], was also conducted for characters under study and illustrated at Table 5. It could be noticed that the mean squares due to genotypes showed significance for number of primary branches and seed yield/ plant, while insignificance for plant height, number of secondary branches and number of pods/ main branch. Therefore, considerable variations among traits expression were detected between white mustard genotypes. Moreover, highly significant variations were obtained detected for number of primary branches, while significant variation for plant height, number of pods/ main branch and seed yield/ plant and insignificant variation for number of secondary branches due to environmental changes.

It was evident that all used models of analysis of variance cleared that there were significant genetic background variations among white mustered genotypes

Table 6a: Estimates of phenotypic stability parameters for plant height of 20 mustard genotypes grown under four diverse environments

Genotypes	x	b_i-ER	S^2d_i-ER	$ -PJ	b_i-FP	S^2d_i-FP	W_i
1	120.575	2.3**	197.64**	1.3	2.41	-579.61	1017.06
2	125.325	1.13	139.23**	0.13	1.19	-598.51	284.91
3	128.325	1.73**	1017.64**	0.73	1.61	-133.93	2229.45
4	115.825	1.14	165.11**	0.14	1.08	-594.76	336.06
5	125.475	1.4*	267.07**	0.40	1.45	-558.04	593.38
6	114.825	1.31*	20.94*	0.31	1.25	-658.28	76.69
7	109.325	2.61**	72.74**	1.61	2.62	-640.59	1090.10
8	115.675	1.88**	154.28**	0.88	2.07	-640.40	595.76
9	104.350	2.19**	210.08**	1.19	2.11	-469.60	941.09
10	119.975	0.98	26.92**	-0.02	0.91	-677.4	53.65
11	123.675	0.36**	60.68**	-0.64	0.20	-643.02	270.98
12	147.500	0.38**	46.54**	-0.62	0.46	-699.46	233.56
13	119.575	0.25**	53.27**	-0.75	0.25	-660.07	310.52
14	132.500	0.26**	115.5**	-0.74	0.46	-636.33	432.85
15	134.225	0.39**	11.16	-0.61	0.25	-692.72	160.55
16	111.925	0.39**	108.94**	-0.61	0.44	-639.37	355.25
17	109.350	0.39**	320.44**	-0.61	0.14	-414.9	787.21
18	138.150	0.29**	172.32**	-0.71	0.33	-575.35	528.93
19	115.575	0.33**	8.18	-0.67	0.29	-703.06	180.66
20	116.000	0.32**	71.21**	-0.68	0.32	-651.54	310.55
LSD 0.05	4.16						
0.01	5.48						

Table 6b: Estimates of phenotypic stability parameters for number of primary branches of 20 mustard genotypes grown under four diverse environments

Genotypes	x	b_i-ER	S^2d_i-ER	$ -PJ	b_i-FP	S^2d_i-FP	W_i
1	15.415	1.38*	0.54	0.38	1.32	-58.12	13.549
2	13.665	1.30	6.28**	0.30	1.24	-55.03	20.427
3	13.165	1.33	7.77**	0.33	1.34	-54.14	24.977
4	13.085	1.53**	1.41	0.53	1.56	-56.92	27.547
5	14.000	0.89	2.18**	-0.11	0.92	-57.58	5.475
6	12.998	1.17	1.05	0.17	1.17	-57.83	4.715
7	11.000	1.22	6.96**	0.22	1.26	-52.91	18.343
8	11.083	1.40*	1.22	0.40	0.97	-57.31	4.143
9	11.583	1.54**	1.41	0.54	1.48	-57.62	28.731
10	12.083	1.15	2.32**	0.15	1.11	-57.24	6.588
11	15.333	0.92	0.76	-0.08	0.97	-57.49	2.154
12	15.748	0.92	4.36**	-0.08	1.07	-53.27	9.235
13	15.920	0.65*	0.69	-0.35	0.56	-57.99	11.913
14	16.000	0.31**	0.51	-0.69	0.28	-57.64	42.450
15	14.665	0.76	0.76	-0.24	0.67	-57.13	6.406
16	15.333	0.73	3.29**	-0.27	0.65	-57.03	12.839
17	15.253	1.08	1.98*	0.08	0.98	-58.09	4.508
18	12.835	0.64*	1.96*	-0.36	0.49	-57.68	15.482
19	12.335	0.61*	0.85	-0.39	0.51	-57.64	15.258
20	12.585	0.72	4.58**	-0.28	0.57	-55.86	15.792
LSD 0.05	1.26						
0.01	1.66						

b_i and S^2d_i: tested against 1.0 and 0.0, respectively, *, ** Denote significant at 0.05 and 0.01 probability levels, respectively
Wi = stability rank of Wricke and Weber [16]

and the response of tested quantitative characters. Also, significant different changes were displayed due to environments. However, all used statistical models confirmed significant genotypes x environmental interaction for most studied traits. These results were in good agreement with those reported by Hasan [23] and Sardana & Borthakur [24].

Data in Table 6 showed that, with the exception of genotypes No. 2, 4 and 10, significant (b_i) values were detected for all other genotypes in plant height. Also, the slope of the regression genotype did not deviate significantly from unity in genotypes No. 11, 12 and 17 for number of primary branches as shown in Table 6a and b.

The deviation from regression mean squares (S^2d_i) were highly significant suggesting that these genotypes were sensitive.

The highest yielding genotypes were No. 1, 2, 3 and 4. The b_i and S^2d_i values were significantly different from unity and zero, respectively for seed yield. Whereas, genotype No. 7 was moderate for seed yield and the (b_i)

Table 6c: Estimates of phenotypic stability parameters for number of secondary branches of 20 mustard genotypes grown under four diverse environments

Genotypes	x	b_i-ER	S^2d_i-ER	$ -PJ	b_i-FP	S^2d_i-FP	W_i
1	4.083	-2.04	0.41**	-3.04	-2.41	-1.73	4.116
2	3.165	0.40	0.14	-0.60	1.11	-1.80	0.399
3	3.248	0.20	0.03	-0.80	0.37	-1.90	0.297
4	3.418	1.4	0.03	0.40	1.30	-1.55	0.109
5	4.668	-2.57**	0.27*	-3.57	-3.89	-1.59	5.074
6	3.665	-0.17	0.33*	-1.17	0.74	-1.77	1.140
7	3.083	0.76	0.05	-0.24	1.85	-1.79	0.118
8	3.333	0.71	0.02	-0.29	0.74	-1.94	0.066
9	3.083	1.48	0.10	0.48	2.59	-1.88	0.276
10	4.085	1.73	0.18	0.73	2.41	-1.73	0.547
11	3.833	2.95**	0.38**	1.95	5.19	-1.43	2.138
12	2.915	1.17	0.13	0.17	2.04	-1.60	0.275
13	2.833	1.84	0.01	0.84	4.07	-1.80	0.273
14	2.915	2.27*	0.01	1.27	4.07	-1.80	0.599
15	2.833	1.12	0.05	0.12	2.04	-1.93	0.116
16	2.750	2.12	0.02	1.12	3.89	-1.93	0.488
17	2.415	1.97	0.03	0.79	2.22	-1.95	0.271
18	2.665	1.74	0.12	0.74	2.41	-1.84	0.452
19	2.498	1.51	0.09	0.51	2.41	-1.84	0.277
20	2.498	1.59	0.05	0.59	3.15	-1.92	0.222
LSD 0.05	0.49						
0.01	0.65						

Table 6d: Estimates of phenotypic stability parameters for number of pods / main branch of 20 mustard genotypes grown under four diverse environments

Genotypes	x	b_i-ER	S^2d_i-ER	$ -PJ	b_i-FP	S^2d_i-FP	W_i
1	33.000	1.47	22.16**	0.47	1.35	-80.96	68.367
2	31.575	1.02	11.65**	0.02	1.16	-91.99	23.311
3	30.525	1.32	11.44**	0.32	1.14	-94.08	34.011
4	29.500	1.30	11.51**	0.30	0.82	-96.69	32.323
5	28.675	0.70*	24.09**	-0.30	0.48	-91.83	57.870
6	30.075	1.32	3.11	0.32	1.23	-96.58	16.736
7	25.600	1.65*	0.60	0.65	1.68	-104.12	46.498
8	26.650	1.55	4.85	0.55	1.46	-100.30	42.286
9	26.650	1.79*	20.74**	0.79	1.66	-81.42	107.750
10	25.825	1.56	5.62	0.56	1.30	-90.26	43.553
11	37.425	1.18	3.84	0.18	1.11	-101.62	11.287
12	31.525	0.66*	4.33	-0.34	0.54	-99.09	20.614
13	29.925	0.61	5.11	-0.39	0.55	-96.54	26.242
14	30.000	0.51	2.89	-0.49	0.63	-104.39	31.014
15	32.075	0.57	31.15**	-0.43	0.60	-92.01	81.198
16	34.475	0.68*	7.25*	-0.32	0.67	-102.21	25.275
17	33.250	0.55	14.39**	-0.45	0.43	-99.87	50.589
18	31.925	0.62	13.95**	-0.38	0.60	-93.61	43.603
19	28.150	0.38	11.03**	-0.62	0.46	-94.34	63.119
20	24.400	0.55	7.38*	-0.45	0.60	-104.07	36.109
LSD 0.05	2.51						
0.01	3.30						

b_i and S^2d_i: tested against 1.0 and 0.0, respectively, *, ** Denote significant at 0.05 and 0.01 probability levels, respectively
Wi = stability rank of Wricke and Weber [16]

value was not significantly different from unity. The minimum deviation from regression mean squares (S^2d_i) pooled over the four environments was obtained by genotypes 7, 13 and 19.

It was concluded that, genotype No. 17 was stable for number of primary branches on the basis of (b_i) which did not differed significantly from unity and ranked second for the mean performance compared with the other genotypes.

The results[5, 26, 27], were more or less in line with these findings.

In addition to high yield, consistency over several environments is much desired for commercial exploitation of the genotype. Wricke's ecovalence model was employed as a stability measurement. This statistic, termed ecovalence (W_i), was simpler to compute and more directly related to genotype-environment interactions. Genotypes with $W_i = 0$ were regarded as perfectly stable.

Table 6e: Estimates of phenotypic stability parameters for seed yield / plant of 20 mustard genotypes grown under four diverse environments

Genotypes	x	b_i-ER	S^2d_i-ER	$\$_{-i}$ -PJ	b_i-FP	S^2d_i-FP	W_i
1	46.175	1.98**	30.61**	0.98	1.65	-138.11	138.47
2	41.525	1.92*	51.64**	0.29	2.04	-134.56	171.48
3	39.400	1.63	7.46*	0.63	1.46	-165.19	46.69
4	38.675	1.72*	2.47	0.72	1.47	-165.79	46.51
5	35.825	1.65	26.00**	0.65	1.25	-149.65	85.74
6	34.000	1.34	3.40	0.34	1.12	-163.33	15.67
7	30.350	1.26	0.28	0.26	1.13	-164.07	6.22
8	33.350	0.37	4.16	-0.63	0.28	-156.20	40.00
9	28.025	0.81	16.35**	-0.19	0.52	-157.69	35.69
10	31.250	0.40	17.62**	-0.60	0.37	-142.77	63.57
11	28.750	0.50	8.53**	-0.50	0.41	-162.63	37.59
12	26.325	0.50	4.81	-0.50	0.42	-165.32	29.67
13	24.575	0.84	0.37	-0.16	0.77	-163.33	2.71
14	25.675	0.62	4.32	-0.38	0.65	-163.54	20.33
15	24.075	0.74	5.77	-0.26	0.60	-159.46	16.97
16	26.925	0.58	8.31**	-0.42	0.44	-160.20	30.97
17	24.675	0.72	9.49**	-0.28	0.53	-157.64	24.88
18	22.175	0.71	6.47*	-0.29	0.64	-159.08	20.24
19	19.175	1.00	0.02	0.00	1.01	-164.19	0.05
20	21.275	0.71	5.97*	-0.29	0.58	-159.48	18.79
LSD 0.05	2.40						
0.01	3.16						

b_i and S^2d_i: tested against 1.0 and 0.0, respectively, *, ** Denote significant at 0.05 and 0.01 probability levels, respectively
Wi = stability rank of Wricke and Weber [16]

Table 7: SDS-PAGE analysis of water soluble and non-soluble proteins of white mustard under variable environments

		Water soluble proteins					Water non-soluble proteins			
		Resources with band density (%)					Resources with band density (%)			
Band no.	MW (kD)	Sandy soil	Sandy soil	Clay soil	Clay soil	MW (kD)	Sandy soil	Sandy soil	Clay soil	Clay soil
1	200	+	+	+	+	112	+++15	+++14	++9	++9
2	138	+	+	+	+	96	+4	+4	++6	++7
3	100	+	+	+	+	90	+	+	+	+
4	99.5	+	+			76	+	+	+	+
5	87	+	+	+	+	66	+	+	+	+
6	81	+	+	+	+	62	+3	+4	++10	++8
7	75	+	+	+	+	48	+3	+3	++7	++6
8	60	+	+	+	+	44	+3	+4	++10	++7
9	58	+	+	+	+	36	++4	++4	+2	+2
10	50	+	+	+	+	35	++4	++5	+3	+3
11	46	+	+	+	+	24	+4	+4	++8	++8
12	42	+	+	+	+	21	+3	+5	++9	++10
13	30	+++19	+++19	++8	++8	15	+++10	+++13	+2	+2
14	20	+++19	+++18	++8	++8	14	++5	++5	+2	+2
15	13	+++11	+++10	++8	++6	9	+++12	++5	+2	+3
16	12	+	+			8			+	+
17						7			++9	++8
18						5	+++21	+++21	+++14	+++12

Such genotypes would not change its performances from one environment to another and probably not exist. According to the meaning of the word "ecovalence" the average stable genotype possesses high ecovalence (low values of W_i = high ecovalence). W_i parameters clearly showed that genotypes No. 19, 13 and 7 considered to be a stable genotypes for seed yield and one or more of the yield attributes recorded (Table 6). Earlier results of Eberhart and Ruseell [12], Perkins and Jinks [13] and Freeman and Perkins [14], are in accordance with these findings.

Oil content %: The oil content of white mustard genotypes increased in sandy soil than clay soil in both

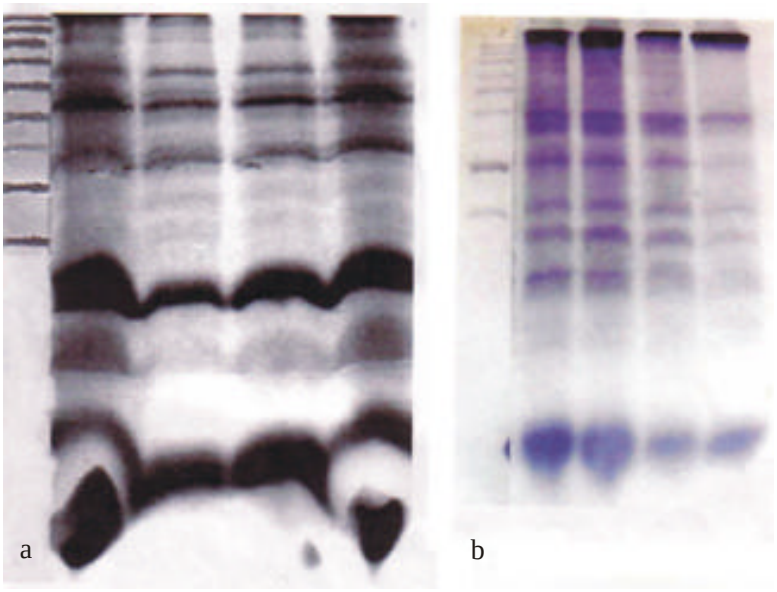

Fig. 1: SDS-PAGE for total soluble proteins of white mustard (a) non-soluble proteins (b)

two growing seasons. These values are (39.26, 42.05%) and (45.01, 49.09%) clay and sandy soil in first and second seasons, respectively.

These data revealed that over all mean values of oil content gave highest values in the second season in sandy soil only.

Genetic characterization of some genotypes of white mustard (*Brassica alba*) by SDS-PAGE analysis: SDS-PAGE of water soluble proteins extracted from four white mustard genotypes revealed that a total of 16 bands with different molecular weights ranged from 200 to 12 kDa in Table 7. Among such protein bands, two bands with molecular weight 99.5 and 12 kDa were presented in the sandy soil, while they were absent in the clay soils in two growing seasons at 2002/ 2003 and 2003/ 2004.

The other protein bands showed that no significant differences upon the presence and the absence of the detected bands. On the other hand, three bands with molecular weights 30, 20 and 13 kDa clearly revealed high density in the sandy soil than in the clay soil with percentages presented in Table 7, which reached more than two fold in most bands.

Among a total of 18 protein bands detected by SDS-PAGE from the water non-soluble fraction, two bands were clearly observed in the clay soils and disappeared in the sandy soil in the two seasons (Fig. 1). Meanwhile, it is interest to note that 13 bands showed outstanding differences based upon the band density in two soils and evidently showed that some minor genes specifically work under a biotic stress (sandy soil) and simultaneously other genes switched off in the same environmental stress. Whereas, seven bands with different molecular weights 112, 36, 35, 15, 14, 9 and 5 showed two fold band density in the sandy soil than in the clay soil. However, other seven bands showed the opposite direction, wherever their band density were much abundant in the clay soil than in the sandy soil Table 7.

In conclusion, the results of SDS-PAGE analysis of proteins of white mustard showed that some new proteins, which were synthesized in response to an altered environment (clay vs. sandy soils) have been obtained as stress proteins, these results are in agreement with many reports Luis *et al.*, [27], Fareida and Afiah [28]. Moreover, some other protein bands represented by their high density percentages were also found much more abundant in the sandy soil than in clay soil.

This finding agreed with Dell' Aquila and Spada [29], El-Enany [30] and Teutonica *et al.*, [31]. They reported that the tolerance to biotic stresses like drought, and salt display a continuous genetic variations because the variation is influenced by simultaneous segregation of several genes.

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Cottonseed, Protein, Oil Yields and Oil Properties as Affected by Nitrogen Fertilization and Foliar Application of Potassium and a Plant Growth Retardant

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Abstract: Field experiments were conducted in two successive seasons, at the Agricultural Research Center, Giza, Egypt. The aim was to investigate the effect of N-fertilization rate (95.2 and 142.8 kg of N/ha, applied as ammonium nitrate containing 33.5% N in two equal doses at 6 and 8 weeks after sowing), together with foliar application of potassium (applied as potassium sulfate containing 48% K_2O at 0.0, 400, 800 or 1200 ppm K_2O, applied twice: 70 and 95 days after sowing) and the plant growth retardant (PGR) Pix (applied twice: 75 days after sowing at 0.0 or 50 ppm and 90 days after sowing at 0.0 and 25 ppm) on seed, protein and oil yields and oil properties of Egyptian cotton cultivar "Giza 86 (*Gossypium barbadense*). The higher N-rate, as well as the application of potassium at different concentrations and plant growth retardant Pix resulted in an increase in cottonseed yield/ha, seed index, seed protein content, oil and protein yield/ha, seed oil refractive index, unsaponifiable matter and total unsaturated fatty acids (oleic and linoleic). Those treatments decreased, though oil acid value, saponification value and total saturated fatty acids. The seed oil content tended to decrease when the high N-rate was applied, but tended to increase with the application of potassium at different concentrations and Pix. There were some differences between potassium concentrations regarding their effects on the studied characters.

Key words: Cottonseed yield % nitrogen % oil fatty acids composition % plant growth retardant % potassium % seed protein content % seed oil content % seed oil properties

INTRODUCTION

Cotton is not only the most important fiber crop in the world, it is also the second best potential source for plant proteins after soybean and the fifth best oil-producing plant after soybean, palm-tree, colza and sunflower [1]. Cotton occupies a prominent position in Egyptian agriculture. It is the main raw material for the largest national industry, the textile industry, as well as the main source of locally produced cottonseed oil. Also, cottonseed meal is classed as a protein supplement in the feed trade. In Egypt, the need to increase the national supply, particularly oil and protein, in quantity and quality is a challenge to agricultural researchers. Oil quality is determined by both nutritional and functional aspects, which are, in turn, primarily determined by the fatty acid profile (i.e., fatty acid composition) of the oil. Economic conditions in modern agriculture demand high crop yields in order to be profitable and consequently match population growth with high demand for food. Crop production can be improved through improving the metabolic activity and nutritional status of crop plants. There are several factors, which can cause such high yields, i.e., development of new high yielding varieties, control of pests and the application of appropriate agronomic practices are potential solutions. Researchers trying some compounds have hormonal effects, while others are nutritions (the proper use of fertilizers, in terms of the quantity and nutrients used and the method of application), which can play and important role in increasing crop production and the quality [2].

In cotton culture, chemical fertilizers, particularly nitrogen (N), are one of the greatest production inputs. Nitrogen is an essential nutrient in creating the plant dry matter, as well as many energy-rich compounds that regulate photosynthesis and plant production [3].

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Synthesis of fat requires both N and carbon skeletons during the course of seed development [4]. The fatty acid composition of seed oil crops is mainly under genetic control, but can affected to some extent by N nutrition [5]. Nitrogen plays the most important role in building the protein structure [6]. Excess N in combination with adequate moisture and high plant populations can increase mutual leaf shading that decreases light intensity in canopy, leading to decrease photosynthate supply and subsequent square shed [7]. Early in development, N deficiency is associated with elevated levels of ethylene, suggesting ethylene production in response to N-deficiency stress [8]. Research in this area has resulted in a decrease in the levels of undesirable long-chain fatty acids. Another beneficial change in fatty acid composition would be an increase in the linoleic and oleic acid contents.

Potassium (K) is one of the most important elements in plant nutrition. All living organisms required in large amounts for normal plant growth and development. This attributed to the role of K in biochemical pathways in plants. Potassium increases the photosynthetic rates of crop leaves, CO_2 assimilation and facilitates carbon movement [9]. Potassium has favorable effects on metabolism of nucleic acids, proteins, vitamins and growth substances [10, 11]. These are manifested in metabolites formed in plant tissues and directly influence the growth and development processes. Furthermore, K has an important role in the translocation of photosynthates from sources to sinks [12]. Notable improvements in cotton yield and quality resulting from K input were reported by Mullins *et al*., [13] and Cassman *et al*., [14]. These may be reflected in distinct changes in seed weight and quality. Pettigrew [15], stated that the elevated carbohydrate concentrations remaining in source tissue, such as leaves, appear to be part of the overall effect of K deficiency in reducing the amount of photosynthate available for reproductive sinks and thereby producing changes in yield and quality seen in cotton.

Several approaches have been tried rise to cotton conductivity. Application of plant growth regulators, particularly growth retardants (PGR) may maintain internal hormonal balance, efficient sink source relationship and thus enhance crop productivity [16]. Mepiquat chloride (Pix) has been found to restrict the vegetative growth and thus enhance reproductive organs [2]. Fan *et al*., [17] indicated that higher photosynthetic efficiency, good population type and canopy structure with dwarf plants, short sympodia, smaller leaves and bigger bolls could be obtained by chemical regulation (Pix).

Table 1: Mechanical and chemical analysis of soil samples

Season	I	II
Mechanical analysis[a]		
Clay (%)	43.00	46.46
Silt (%)	28.40	26.38
Fine sand (%)	19.33	20.69
Coarse sand (%)	4.31	1.69
Texture	Clay loam	Clayloam
Chemical analysis[b]		
Organic matter (%)	1.83	1.92
Calcium carbonate (%)	3.00	2.73
Total soluble salts (%)	0.13	0.13
pH (1:2.5)	8.10	8.08
Total nitrogen (%)	0.12	0.12
Available nitrogen (mg/kg soil)	50.00	57.50
Available phosphorus (mg/kg soil)	15.66	14.19
Available potassium (mg/kg soil)	370.00	385.00
Calcium (meq/100 g)	0.20	0.20

[a]According to Kilmer and Alexander [18],

[b]According to Chapman and Pratt [19],

Note: The field was divided into uniform soil areas; eight soil samples to plow depth 30 cm were collected at random over the field and mixed to give a composite sample

In the present study, an attempt was made to investigate the effects of nitrogen fertilization rate and foliar application of potassium and Pix, during square initiation and boll setting stage on cottonseed, protein and oil yields and on oil properties and fatty acid profiles of oil in the seed of Egyptian cotton (*Gossypium barbadense* L., cv. Giza 86), because a suitable management practice for application of N, K and Pix to optimize these traits has not yet been developed.

MATERIALS AND METHODS

Two field experiments were conducted at the Agricultural Research Center, Ministry of Agriculture in Giza (30°N, 31° :28'E and 19 m altitude), Egypt using the cotton cultivar "Giza 86" (*Gossypium barbadense* L.) in two successive seasons. The soil type in both seasons was a clay loam. Average mechanical analysis [18] and chemical characteristics [19], for soil in both seasons is illustrated in Table 1. Each experiment included 16 treatment combinationsof the following: (i) Two nitrogen rates (95.2 and 142.8 kg of N/ha) were applied as ammonium nitrate with lime (NH_4NO_3 + $CaCO_3$, 33.5% N) at two equal doses, 6 and 8 weeks after sowing. Each application (in the form of pinches beside each hill) was followed immediately by irrigation. (ii) Four K rates (0.0, 400, 800 and 1200 ppm of K_2O) were applied as potassium sulfate (K_2SO_4, "48% K_2O"). Each was foliar sprayed twice, 70 and 95 days after planting (during square initiation and bolling stage) and the solution volume was 960 L/ha. (iii) Two rates from the PGR, 1,1-dimethylpiperidinium chloride (mepiquat chloride or

Table 2: Treatments number and summary

Treatment No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
N rate (kg/ha)	95.2	95.2	95.2	95.2	95.2	95.2	95.2	95.2	142.8	142.8	142.8	142.8	142.8	142.8	142.8	142.8
K_2O rate (ppm)	0.0	0.0	400	400	800	800	1200	1200	0.0	0.0	400	400	800	800	1200	1200
Pix rate (ppm)	0.0	50 & 25	0.0	50 & 25	0.0	50 & 25	0.0	50 & 25	0.0	50 & 25	0.0	50 & 25	0.0	50 & 25	0.0	50 & 25

"Pix") were foliar sprayed twice, (75 days after planting at 0.0 or 50 ppm and 90 days after planting at 0.0 and 25 ppm), where the solution volume was also 960 L/ha. The K_2O and PGR were both applied to the leaves with uniform coverage using a knapsack sprayer. The pressure used with the sprayer utilized in the study was 0.4 kg/cm^2, resulting in a nozzle output of 1.43 L/min. The application was carried out between 09.00 and 11.00 h. A summary of all treatments is shown in Table 2.

A randomized complete block design with four replications was used. Seeds were planted in plots 1.95 m x 4.0 m. (after the precaution of border effect was taken into consideration) on April 3rd and 8th in the first and second seasons, respectively. The size consisted of three ridges. Hills were spaced at 25 cm apart on one side of the ridge and seedlings were thinned to two plants/hill 6 weeks after planting, providing plant density of 123,000 plants/ha. Total irrigation amount during the growing season (surface irrigation) was about 6,000 m^3/ha. The first irrigation (after sowing irrigation) was applied 3 weeks after sowing and the second one was 3 weeks later. Thereafter, the plots were irrigated every 2 weeks until the end of the season, thus providing a total of nine irrigations. On the basis of soil test results, phosphorus fertilizer was applied at the rate of 54 kg P_2O_5 ha^{-1} as calcium superphosphate during land preparation and potassium fertilizer was applied at the rate of 57 kg K_2O ha^{-1} as potassium sulphate before the first irrigation (the recommended level for semi-fertile soil). Pest and weed management were carried out during the growth season, according to local practice performed at the experimental station.

Total cotton yield/plot was determined by first hand-picking on September 20 and 27 with final picking on October 5 and 12 in the first and second seasons, respectively. At harvest, total cotton yield/plot was determined. Following ginning, the cottonseed yields were determined in kg/ha, along with seed index weight in g/100 seeds. Seed samples of the four replicates/treatment were combined for chemical analyses. The following chemical analyses were conducted: (i) seed crude protein content according to AOAC standards [20]; (ii) seed oil content in which oil was extracted three times with chloroform/methanol (2:1, vol/vol) mixture according to the method outlined by Kates [21]; (iii) oil quality traits, i.e., refractive index, acid value, saponification value, unsaponifiable matter and iodine value were determined according to methods described by AOCS [22] and (iv) identification and determination of oil fatty acids by gas-liquid chromatography. The lipid materials were saponified, unsaponifiable matter was removed and the fatty acids were separated after acidification of the saponifiable materials. The free fatty acids were methylated with diazomethane [23]. The fatty acid methyl esters were analyzed by a Hewlett Packard model 5890 gas chromatograph (Palo Alto, CA) equipped with dual flame-ionization detectors. The separation procedures were similar to those reported by Ashoub *et al*., [24] as follows: The chromatograph was fitted with an FFAP (crosslinked) 30 m (length) x 0.32 mm (column i.d.) x 0.25 µm (film thickness) capillary column coated with polyethylene glycol. The column oven temperature was programmed at 7°C/min from 50 to 240°C and kept finally to 30 min. Injector and detector temperatures were 250 and 260°C, respectively. Gas flow rates were 33, 30 and 330 mL/min for N_2, H_2 and air, respectively, with N_2 flow rate inside column of 2 mL/min. Under these conditions, all peaks from C8 to 20 homologous series were well defined. Peak identification was performed by comparison of the relative retention time (RRT) for each peak with those of standard chromatograms. The RRT of oleic acid was given a value of 1.0. Results were expressed as an area percentage of chromatograms.

Statistical analysis: Data obtained for the cottonseed yield and seed index were statistically analyzed factorially according to procedures outlined by Snedecor and Cochran [25] and the least significant difference (LSD) was used to determine the significance of differences between treatment means at 0.05 level. As for the chemical properties considered in the study, the t-test computed in accordance with standard deviation was utilized to verify the significance between every two-treatment means at the 0.05 level of significance.

RESULTS AND DISCUSSION

Cottonseed yield: Seed yield per hectare significantly ($p<0.05$) increased (as much as 13.03%) due to raising the

Table 3: Effect of N rate and foliar application of K and a plant growth retardant "Pix" on cottonseed yield, seed index, seed oil, seed protein, oil and protein yields

Treatments	Cottonseed yield (kg/ha)[a]	Seed index (g)[a]	Seed oil (%)[b]	Oil yield (kg/ha)[b]	Seed protein (%)[b]	Protein yield (kg/ha)[b]
N-rate (kg/ha)						
95.2	1862.4	10.09	19.73	367.5	22.24	414.2
142.8	2105.0[d]	10.32[d]	19.60	413.0[d]	22.44[d]	472.2[d]
LSD 0.05[c]	78.78	0.075	-	-	-	-
SD[c]	-	-	0.167	33.65	0.113	35.5
K_2O-rate (ppm)						
0, control	1804.4	10.03	19.49	351.6	22.32	402.9
400	1985.2[d]	10.19[d]	19.61	389.3[d]	22.32	443.1
800	2047.7[d]	10.27[d]	19.73[d]	404.2[d]	22.34	457.7[d]
1200	2097.6[d]	10.32[d]	19.83[d]	415.8[d]	22.37	469.3[d]
LSD 0.05[c]	111.41	0.106	-	-	-	-
SD[c]	-	-	0.129	35.06	0.165	41.87
Pix-rate (ppm)						
0, control	1891.8	10.13	19.61	371.1	22.31	422.1
50 & 25	2075.6[d]	10.27[d]	19.72	409.4[d]	22.37	464.4[d]
LSD 0.05[c]	78.78	0.075	-	-	-	-
SD[c]	-	-	0.170	36.11	0.151	41.35

[a]Combined statistical analysis from the two seasons, [b]Mean data from a four replicate composites for the two seasons,
[c]LSD = Least significant differences, SD = Standard deviation was used to conduct t-test to verify the significance between every two treatment means at 0.05 level, [d]Significant at 0.05 level

N-rate from 95.2 to 142.8 kg of N/ha (Table 3). This could be attributed to the fact that N is an essential nutrient in creating the plant dry matter, as well as many energy-rich compounds which regulate photosynthesis and plant production [3]. There is an optimal relationship between the nitrogen content in the plant and CO_2 assimilation [26], where decreases in CO_2 fixation are well documented for N-deficient plants. Nitrogen deficiency is associated with elevated levels of ethylene (which increase boll shedding), suggesting ethylene production in response to N-deficiency stress [8]. These results agreed with those obtained by Brar *et al*., [27], when N was applied up to 150 kg/ha and Ram *et al*., [28], when N was applied up to 100 kg/ha.

Potassium application significantly increased cottonseed yield per hectare, where the three concentrations applied (400, 800 and 1200 ppm of K_2O) proved to excel the control (by 10.02-16.25%). Nevertheless, the differences between the effects of the three concerned K_2O rates were statistically insignificant; with the exception of the 1200 ppm concentration which proved to produce significantly higher cottonseed yield per hectare (5.66%) than the 400 ppm concentration. In general, it could be stated that the highest K_2O concentration (1200 ppm) was numerically better than the other two concentrations (400 or 800 ppm). These increases could be due to favorable effects of this nutrient on yield components such as number of opened bolls/plant, boll weight, or both, leading to higher cotton yield. Zeng [29] indicated that, K fertilizer reduced boll shedding. Pettigrew [15] stated that, the elevated carbohydrate concentrations remaining in source tissue, such as leaves, appear to be part of the overall effect of K deficiency in reducing the amount of photosynthate available for reproductive sinks and thereby producing changes in boll weight. Cakmak *et al*., [12] found that, potassium nutrition had pronounced effects on carbohydrate partitioning by affecting either phloem export of photosynthates (sucrose) or growth rate of sink and/or source organs. Mullins *et al*., [30] evaluated cotton (*Gossypium hirsutum*) yield under a long-term surface application of K at 60-180 lb K_2O/acre and found that K application increased yield. The obtained results confirmed those of Howard *et al*., [31] and Gormus [32].

Application of the plant growth retardant Pix significantly increased seed yield per hectare (by 9.72%), as compared with untreated plants. Such increases could be due to that, application of Pix may maintain internal hormonal balance, efficient sink source relationship and thus enhance crop productivity [16]. Pix have been found to restrict the vegetative growth and thus enhance reproductive organs by allowing plants to direct more energy towards the reproductive structure [2]. Also, such increases may be due to increase photosynthetic activity of leaves when this substance is applied [33]. This means that bolls on treated cotton plants would have a larger photosynthetically-supplied sink of carbohydrates and other metabolites than did those on untreated cotton plants. Results agreed with those obtained by Mekki [34], when Pix was applied at 100 ppm and Ram *et al*., [28], when Pix was applied at 50 ppm.

Seed index: Seed index significantly increased by adding the high N-rate (Table 3). This may be due to increased photosynthetic activity which increases accumulation of metabolites, with direct impact on seed weight. Reddy *et al*., [35], in a pot experiment under natural environmental conditions, where 20-day old cotton plants received 0, 0.5, 1.5 or 6 mM NO_3, found that, net photosynthetic rates, stomatal conductance and transpiration were positively correlated with leaf N concentration. Similar findings were reported by Palomo *et al*., [36], when N was applied at 40-200 kg/ha and Ali and El-Sayed [37], when N was applied at 95-190 kg/ha.

Seed index significantly increased with K application at all the three concentrations, over the control. The highest rate of K_2O (1200 ppm) showed the highest numerical value of seed index although it differ significantly from the value of the lower rate (400 ppm). Possible explanation for increasing seed index due to the application of K may be due in part to its favorable effects on photosynthetic activity rate of crop leaves [11] and CO_2 assimilation, which improves mobilization of photosynthates and directly influences boll weight that directly effect seed weight. This finding corroborated the results obtained by Sabino *et al*., [38] and Ghourab *et al*., [39]. Application of Pix, significantly increased seed index over the untreated control. Increased seed weight as a result of Pix applications may be due to increase in photosynthetic activity which stimulates photosynthetic activity and dry matter accumulation [33] and in turn increases formation of fully-mature seed and thus increases seed weight. This finding was in good accordance with those obtained by Mekki [34].

Seed oil content and yield: Seed oil content was slightly decreased when additional N was applied, but oil yield per hectare (total production) significantly increased (by 45.5 kg oil/ha), which is attributed to the significant increase in cottonseed yield (Table 3). Nitrogen is an essential nutrient in creating the plant dry matter, as well as many energy-rich compounds which regulate photosynthesis and plant production [3], thus influencing boll development, increasing the number of bolls/plant and boll weight. Synthesis of fat requires both N and carbon skeletons during the course of seed development [4]. Similar results were obtained by Froment *et al*., [40], in linseed and Zubillaga *et al*., [41], in sunflower.

Application of K at all the three concentrations tended to increase numerically the seed oil content and oil yield per hectare over the control (by 37.7-64.2 kg oil/ha), but was statistically significant only for 800 and 1200 ppm K_2O concentrations on the seed oil content and with K application at all the three concentrations on the oil yield per hectare. The highest rate of K (1200 ppm K_2O) showed the highest numerical values of seed oil content and oil yield per hectare compared with the other two concentrations (400 and 800 ppm K_2O). This could be attributed to the role of K in biochemical pathways in plants. Pettigrew [15] stated that, the elevated carbohydrate concentrations remaining in source tissue, such as leaves, appear to be part of the overall effect of K deficiency in reducing the amount of photosynthate available for reproductive sinks and thereby producing changes in yield and quality seen in cotton. Madraimov [42] indicated that, increasing the rates of applied K_2O from 0 to 150 kg/ha produced linear increases in cottonseed oil contents. Previously, favorable effects of K on seed oil content and oil yield were mentioned by Fan *et al*., [17] and Abou El-Nour *et al*., [43]. They reported that increasing K supply to maternal cotton plants increased crude fat content of seed.

Application of Pix resulted in an insignificant increase in seed oil content over that of the control. Also significantly increased the seed oil yield per hectare compared with the untreated control (by 38.3 kg oil/ha). These results could be attributed to the increase of total photoassimilates (e.g., lipids) and the translocated assimilates to the sink as a result of applying Pix [17]. This result agreed with those obtained by Mekki and El-Kholy [44], in rape.

Seed protein content and yield: High N-rate increased significantly the seed protein content and yield per hectare (by 58.0 kg protein/ha) (Table 3). Stitt [45] indicated that nitrate (NO_3G) induces genes involved in different aspects of carbon metabolism, including the synthesis of organic acids used for amino acid synthesis. These results suggest that the high N rate increases the amino acids synthesis in the leaves and this stimulate the accumulation of protein in the seed. The present results confirmed the findings of Patil *et al*., [46].

Potassium tended to increase insignificantly the seed protein content compared with untreated control, when applied at 800 and 1200 ppm K_2O. Applied K at all rates also, increased numerically the protein yield per hectare (by 40.2-66.4 kg protein/ha), resulting from an improvement in both cottonseed yield and seed protein content. The increase in protein yield per hectare was statistically significant when applied the 800 and 1200 ppm K_2O concentrations. Best protein yield was obtained at the high K concentration (1200 ppm K_2O)

Table 4: Effect of N rate and foliar application of K and a plant growth retardant "Pix" on seed oil properties[a]

Treatments	Refractive index	Acid value	Saponification value	Unsaponifiable matter (%)	Iodine value
N-rate (kg/ha)					
95.2	1.4684	0.1339	190.84	0.3762	128.89
142.8	1.4695	0.1313[c]	189.74	0.3913	131.14
SD[b]	0.00118	0.00259	1.453	0.01786	3.349
K_2O-rate (ppm)					
0, control	1.4682	0.1352	190.78	0.3675	125.79
400	1.4689	0.1337	190.06	0.3825	130.30[c]
800	1.4692	0.1315[c]	190.25	0.3875[c]	131.59[c]
1200	1.4694	0.1300[c]	190.07	0.3975[c]	132.39[c]
SD[b]	0.00129	0.00217	1.526	0.01707	2.468
Pix-rate (ppm)					
0, control	1.4683	0.1331	190.62	0.3750	128.28
50 & 25	1.4696[c]	0.1321	189.96	0.3925[c]	131.75[c]
SD[b]	0.00110	0.00289	1.658	0.01721	3.036

[a]Mean data from a four replicate composites for the two seasons, [b]SD = Standard deviation, [c]Significant at 0.05 level

compared with the other two concentrations (400 and 800 ppm K_2O). This could be attributed to the role of K in biochemical pathways in plants. Potassium has favorable effects on metabolism of nucleic acids and proteins[10, 11]. These are manifested in metabolites formed in plant tissues and directly influence the growth and development processes and thereby producing changes in yield and quality seen in cotton. These results were in good agreement with those obtained Abou El-Nour *et al*., [43] and Ghourab *et al*., [39].

Seed protein content was increased insignificantly, while seed protein yield per hectare was significantly increased (by 42.3 kg protein/ha) in plants treated with Pix compared with the untreated control. The increase in seed protein content may be caused by the role of Pix in protein synthesis, encouraging the conversion of amino acids into protein [47]. Also, for the favorable and significant effect of Pix on cottonseed yield. These results were confirmed by Abdel-Al *et al*., [48].

There was no clear relationship between protein and oil, in this which may be due to low application doses not sufficiently great enough to allow expression of the expected inverse relationship between oil and protein.

Seed oil properties: The seed oil refractive index, unsaponifiable matter and iodine value tended to increase, while the oil saponification value and acid value tended to decrease insignificantly by raising N-rate (Table 4). The increase in unsaponifiable matter is beneficial as it increases the oil stability. Narang *et al*., [49] indicated that, N application increased the oil-quality index (iodine number) in rape.

Application of K at different concentrations tended to increase the seed oil refractive index, unsaponifiable matter and iodine value and to decrease the oil saponification value and acid value, numerically, compared with the untreated control, especially when applied K at the high concentration (1200 ppm K_2O). The effect was significant for the two concentrations 800 and 1200 ppm K_2O on acid value and unsaponifiable matter and for all different concentrations on iodine value. The effect of K_2O-concentrations on oil refractive index were very limited. Potassium is an essential nutrient and an integral component of several important compounds in plant cells. This attributed to the role of K in biochemical pathways in plants, where K acts as an activator for several enzymes involved in carbohydrates metabolism [50]. These may be reflected in distinct changes in seed oil quality. Mekki *et al*., [51] stated that, foliar application with K (0 or 3.5% K_2O) on sunflower at the seed-filling stage, decreased oil acid value. Froment *et al*., [40] in linseed found that, the iodine value, which indicates the degree of unsaturation of the final oil, was highest in treatment receiving extra K.

Application of Pix tended to increase significantly the oil refractive index, unsaponifiable matter and iodine value, while tended to decrease insignificantly the oil acid value and saponification value, compared with the untreated control. Application of plant growth regulators, particularly growth retardants may maintain internal hormonal balance and efficient sink source relationship [16]. This may be reflected in distinct changes in seed oil quality.

Oil fatty acids composition: The oil saturated fatty acids, lauric, myristic, palmitic and the total ones decreased, while capric and stearic increased by raising N-rate (Table 5). The effect was significant only on palmitic acid, which was the dominant saturated fatty acid. Low content of saturated fatty acids is desirable for edible uses. The total unsaturated fatty acids (oleic and linoleic) and the ratio between total unsaturated fatty acids and total

Table 5: Effect of N rate and foliar application of K and a plant growth retardant "Pix" on the relative percentage of saturated fatty acids[a]

	Relative % of saturated fatty acids					
Treatments	Capric	Lauric	Myristic	Palmitic	Stearic	Total
N-rate (kg/ha)						
95.2	0.0684	0.0680	0.6912	21.77	2.157	24.7526
142.8	0.0691	0.0666	0.6450	20.18[c]	2.969	22.9345
SD[b]	0.00929	0.00649	0.45113	1.446	0.4705	2.28338
K_2O-rate (ppm)						
0, control	0.0775	0.0745	1.3075	22.40	2.602	26.4670
400	0.0722	0.0698[c]	0.6750[c]	21.02	1.955[c]	23.7920[c]
800	0.0648[c]	0.0632[c]	0.3500[c]	20.52[c]	1.905[c]	22.9030[c]
1200	0.0605[c]	0.0618[c]	0.3400[c]	19.96[c]	1.790[c]	22.2122[c]
SD[b]	0.00659	0.00384	0.17971	1.477	0.3690	1.92554
Pix-rate (ppm)						
0, control	0.0739	0.0655	0.7750	21.97	2.336	25.2206
50 & 25	0.0636[c]	0.0691	0.5612	19.98[c]	1.790[c]	22.4665[c]
SD[b]	0.00752	0.00623	0.43717	1.296	0.3826	1.99777

[a]Mean data from a four replicate composite for the two seasons, [b]SD = Standard deviation, [c]Significant at 0.05 level

Table 6: Effect of N rate and foliar application of K and a plant growth retardant "Pix" on the relative percentage of unsaturated fatty acids[a]

	Relative % of unsaturated fatty acids			TU/TS[b]
Treatments	Oleic	Linoleic	Total	ratio
N-rate (kg/ha)				
95.2	21.59	53.65	75.24	3.069
142.8	22.99[d]	54.08	77.06	3.397
SD[c]	1.353	1.144	2.284	0.4030
K_2O-rate (ppm)				
0, control	21.26	52.26	73.53	2.790
400	22.11	54.10[d]	76.20[d]	3.228[d]
800	22.60	54.50[d]	77.09[d]	3.390[d]
1200	23.18	54.60[d]	77.78[d]	3.523[d]
SD[c]	1.370	0.634	1.925	0.3519
Pix-rate (ppm)				
0, control	21.27	53.51	74.77	2.984
50 & 25	23.31[d]	54.22	77.53[d]	3.482[d]
SD[c]	1.095	1.102	1.998	0.3496

[a]Mean data from a four replicate composite for the two seasons,
[b]TU/TS ratio = (total unsaturated fatty acids) / (total saturated fatty acids)
[c]SD = Standard deviation, [d]Significant at 0.05 level

saturated fatty acids (TU/TS) were increased (by 2.42 and 10.69%, respectively) by raising N-rate (Table 6). The effect was significant only on oleic acid. Linoleic acid was the most abundant unsaturated fatty acid. Holmes and Bennett [5] commented that, the fatty acid composition of rape oil is mainly under genetic control, but can be modified to some extent by N nutrition. Seo *et al*., [52] found that, when sesame was given 0-160 kg N, oleic acid content was highest at the highest N rates and linoleic acid content was highest at the intermediate rates. Khan *et al*., [53] indicated that, oleic acid increased by increasing levels of N added to rapeseed-mustard. Kheir *et al*., [54], in flax, found that the higher N-rate increased the percentage of unsaturated fatty acids and decreased saturated fatty acids in the seed oil.

Potassium applied at all concentrations resulted in a decrease in the total saturated fatty acids (capric, lauric, myristic, palmitic and stearic) compared with untreated control. Spraying plants with the high K concentration 1200 ppm K_2O gave the lowest total saturated fatty acids oil, compared with the other two concentrations (400 and 800 ppm). The effect was significant for the two concentrations 800 and 1200 ppm K_2O on capric and palmitic and for all different concentrations on lauric, myristic, stearic and the total saturated fatty acids. Potassium applied at all rates increased the total unsaturated fatty acid (oleic and linoleic) and TU/TS ratio (by 1.84-4.48 and 15.70-26.27%, respectively), compared with untreated control. Applied K at 1200 ppm K_2O gave the highest increment, followed by 800 ppm concentration. The effect was significant for all different concentrations on linoleic, the total unsaturated fatty acid and TU/TS ratio. Linoleic acid was the most abundant unsaturated fatty acid. The beneficial effect of applied K on TU and TU/TS ratio suggests that might be due to the regulated effect of K which acts as an activator on many enzymic processes, where some of these enzymes may affect the seed oil content from these organic matters. Seo *et al*., [52] found that, when sesame was given 0-180 kg K_2O, oleic acid content was the highest at the highest K rates and linoleic acid content was the highest at the intermediate rates. Salama [55] indicated that, K fertilizer applied to sunflower cv. IH-173, favoured fatty acid composition (high oleic acid content). Mekki *et al*., [51] stated that, foliar application with K on sunflower increased the oleic acid fatty acid. Froment *et al*., [40] in linseed oil found that, linoleic acid content was greatest in treatment receiving extra K.

Application of Pix resulted in a decrease in the total saturated fatty acids, the abundant saturated fatty acid palmitic, capric, myristic and stearic while resulted in an increase in lauric saturated fatty acid, compared to untreated control. The effect was significant only on capric, palmitic, stearic and the total ones. Application of Pix resulted in an increase in total unsaturated fatty acids (oleic and linoleic) and TU/TS ratio (by 3.69 and 16.69%, respectively), over the control. The effect was significant only on the total unsaturated fatty acid, oleic and the ratio between total unsaturated fatty acids and total saturated fatty acids (TU/TS). The stimulatory residual effects of application Pix on TU and TU/TS ratio was probably due to its favourable effects on fundamental metabolic reactions in plant tissues and would have direct impact through utilization in growth processes which are reflected in distinct changes in seed oil quality. Some of these changes may affect the seed oil fatty acids composition, which may attribute to their encouraging effects on enzymes that catalyzed the biosynthesis of the unsaturated fatty acids. Mekki and El-Kholy [44] investigate the response of oil seed rape to 0, 200 or 400 ppm Pix and found that; palmitic acid was only decreased by using 400 ppm Pix as compared with 200 ppm treatmrnt or control plants.

Low content of saturated fatty acids is desirable for edible uses. Also, regarding oil quality, higher levels of linoleic acid and oleic acid are considered good for oil quality [56].

During the two growing seasons no significant interactions were found between the variables in the present study (N-rate and foliar application of K and the plant growth retardant Pix) on quantitative and qualitative characters under investigation. Regarding insignificant interaction effects, the F ratios worthy exceed unity, but within the level of probability take p#0.05, they did not show significance.

CONCLUSIONS

From the findings of the present study, it seems rational to recommend application of N at a rate of 142.8 kg/ha, combined with spraying cotton plants with K twice (especially K_2O-concentration of 1200 ppm) and application of Pix, also twice (at a rate of 50 and 25 ppm, respictively). These treatments, would beneficially affect not only the quantity (to obtain higher oil and protein yields) but also the quality of oil (as indicated by better fatty acid profile in the oil of cotton) of cottonseed. In comparison with the ordinary cultural practices adopted by Egyptian cotton producers, it is quite apparent that applications such treatments could bring about better impact on cottonseed yield, seed protein content, oil and protein yields, oil refractive index, unsaponifiable matter, iodine value and unsaturated fatty acids. On the other hand, there was a decrease in acid oil value and saponification value.

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Cottonseed, Protein, Oil Yields and Oil Properties as Influenced by Potassium Fertilization and Foliar Application of Zinc and Phosphorus

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Abstract: In maximizing the yield quantity and quality of a crop in terms of the nutritional value of fatty acids and protein, it is necessary to identify the constraints, which operate at a site and to devise methods of overcoming them through the use of inputs or changes in management practices. Field experiments were conducted during two successive seasons at the Agricultural Research Center, Giza, Egypt, on the cotton cultivar "Giza 86" (*Gossypium barbadense* L.) to study the effects of potassium fertilization (at 0.0 and 57.1 kg of K_2O/ha) and foliar application of zinc (at 0.0 and 60 ppm of Zn, two times, 70 and 85 days after planting, "during square initiation and boll setting stage") and phosphorus (at 0.0, 600, 1200 and 1800 ppm of P_2O_5, two times, 80 and 95 days after planting) on cottonseed. Application of potassium and spraying plants with zinc and phosphorus caused an increase in cottonseed yield/ha, seed index, seed oil content, oil and protein yields/ha, seed oil unsaponifiable matter and total unsaturated fatty acids (oleic and linoleic). However, those treatments resulted in a decrease in oil acid value, saponification value and total saturated fatty acids. The highest P_2O_5-concentration of 1800 ppm gave the best values of cottonseed yield/ha, seed index and seed oil and protein yields/ha and oil saponifiable matter.

Key words: Cottonseed % oil fatty acids % oil properties % phosphorus % potassium % zinc

INTRODUCTION

In Egypt, the need to increase the national supply, particularly oil and protein, in quantity and quality must be among the list of goals of cotton breeders and producers. Agricultural scientists believe that this challenge can be met. It is expected that plant nutrition, using a balanced fertilization program with both macro and micro-nutrients has become, very important in the production of high yield with high quality products specially with the large variation fertility of soil and crop's needs for macro- and micro-nutrients. The breeding and production of cotton have traditionally been guided by consideration of fiber quality and yield. However, cottonseed characteristics except for viability and vigour have generally been ignored. Cottonseed oil is an important source of fat. Also, cottonseed meal is classed as a protein supplement in the feed trade; it is almost as important as soybean meal. Therefore, the need to increase oil and protein content must be among the list of goals of cotton breeders and producers. Potassium, zinc and phosphorus are three essential nutrients for crop production and crop reproduction [1-3].

Potassium (K) is an essential macro-element required in large amounts for normal plant growth and development. This attributed to the role of K in plant biochemical pathways [4]. Potassium increases the photosynthetic rates of crop leaves, CO_2 assimilation and facilitates carbon movement [5]. Furthermore, K has an important role in the translocation of photosynthates from sources to sinks [6]. Pettigrew [1] stated that the elevated carbohydrate concentrations remaining in source tissue, such as leaves, appear to be part of the overall effect of K deficiency in reducing the amount of photosynthate available for reproductive sinks and thereby producing changes in yield and quality seen in cotton. Studies have shown increased yield and quality of cotton in response to K fertilization. Notable improvements in cotton yield and quality resulting from K input may be reflected in distinct changes in seed weight and quality.

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Crop yields are often limited by low soil levels of mineral micronutrients such as zinc (Zn), especially in calcareous soils of arid and semiarid regions. Zinc deficiency occurs in cotton on high-pH soils, particularly where topsoil has been removed in preparing fields for irrigation and thereby exposing the Zn-deficient subsoil. Also, Zn deficiencies have occurred where high rates of P are applied [7]. Zinc is an essential mineral nutrients and a cofactor of over 300 enzymes and proteins involved in cell division, nucleic acid metabolism and protein synthesis [8]. Zinc is an component of a number of dehydrogenases, proteinases and peptidases; thus Zn influences electron transfer reactions including those of the Krebs cycle and hence affecting the plant's energy production. Zinc binding tightly to Zn-containing essential metabolites in vegetative tissues, such as in Zn-activated enzymes, e.g., carbonic anhydrase, which plays a role in photosynthesis, is localized in the cytoplasm and chloroplasts and may facilitate the transfer of CO_2/HCO_3G for photosynthetic CO_2 fixation [2]. Further, Zn is required in the biosynthesis of tryptophan, a precursor of the auxin indole-3-acetic acid (IAA) [7], which is the major hormone that inhibits abscission of squares and bolls.

Phosphorus (P) has been found to be the life-limiting element in natural ecosystems because it is often bound in highly insoluble compounds and hence it becomes unavailable for plant uptake or utilization. High soil pH (>7.6) and high quantities of $CaCO_3$ result in precipitation of P, which reduces the soluble P supply [9]. Phosphorus is an essential nutrient and an integral component of several important compounds in plant cells, including the sugar-phosphates involved in respiration, the phospholipids of plant membranes and the nucleotides used in plant energy metabolism and in molecules of DNA and RNA [3]. Rodriguez *et al*., [10] observed that in a P deficiency, there was a reduction in the rate of leaf expansion and in photosynthetic rate per unit of leaf area. Phosphorus, as a constituent of the cell nucleus, is essential for cell division and development of meristematic tissue [11]. Moreover, P plays a decisive role in carbon assimilate transport and metabolic regulation (starch, sucrose biosynthesis) on a whole-plant scale [12]. Further, it has a well-known impact on photosynthesis as well as synthesis of nucleic acids, proteins, lipids and other essential compounds [3].

Due to the economic importance of cottonseed as the main source of edible oil for human consumption and meal for live-stock in Egypt, this study was designed to determine the extent of improvement in cottonseed, protein and oil yields and on oil properties and fatty acid profiles of oil in the seed of Egyptian cotton (*G. barbadense*) as affected by potassium fertilization and foliar application of chelated zinc and phosphorus, during square initiation and boll setting stage.

Table 1: Mechanical and chemical analysis of soil samples

Season	I	II
Mechanical analysis[a]		
Clay (%)	43.00	46.46
Silt (%)	28.40	26.38
Fine sand (%)	19.33	20.69
Coarse sand (%)	4.31	1.69
Texture	Clay loam	Clay loam
Chemical analysis[b]		
Organic matter (%)	1.83	1.92
Calcium carbonate (%)	3.00	2.73
Total soluble salts (%)	0.13	0.13
pH (1:2.5)	8.10	8.08
Total nitrogen (%)	0.12	0.12
Available nitrogen (mg/kg soil)	50.00	57.50
Available phosphorus (mg/kg soil)	15.66	14.19
Available potassium (mg/kg soil)	370.00	385.00
Available zinc (mg/kg soil)	1.30	1.90
Calcium (meq/100 g)	0.20	0.20

[a]According to Kilmer and Alexander [13],

[b]According to Chapman and Pratt [14], Note: The field was divided into uniform soil areas; eight soil samples to plow depth 30 cm were collected at random over the field and mixed to give a composite sample

MATERIALS AND METHODS

Two field experiments were conducted at the Agricultural Research Center, Ministry of Agriculture in Giza (30°N, 31°:28'E and 19 m altitude), Egypt on the cotton (*Gossypium barbadense* L.) cultivar "Giza 86", in two successive seasons. The soil type in both seasons was a clay loam. Average mechanical analysis [13] and chemical characteristics [14] of soil in both seasons are recorded in Table 1. Each experiment included 16 treatment combinations of the following: (i) Two potassium rates (0.0 and 57.1 kg of K_2O/ha) were applied as potassium sulfate (K_2SO_4, "48% K_2O), eight weeks after sowing (as a concentrated band close to the seed ridge) and the application was followed immediately by irrigation. (ii) Two zinc rates (0.0 or 60 ppm of Zn) were applied as chelated form [ethylenediaminetetraacetic acid (EDTA)] and each was foliar sprayed two times (70 and 85 days after sowing, during square initiation and bolling stage). (iii) Four phosphorus rates (0.0, 600, 1200 and 1800 ppm of P_2O_5) were applied as calcium superphosphate (15% P_2O_5) and each was foliar sprayed two times (80 and 95 days after sowing). The Zn and K_2O were both applied to the leaves with uniform coverage at a volume solution of 960 L/ha, using a knapsack sprayer. The pressure used with the sprayer utilized in the study was 0.4 kg/cm^2, resulting in a nozzle output of 1.43 L/min.

Table 2: Treatments number and summary

Treatment No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
K_2O rate (kg/ha)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	57.1	57.1	57.1	57.1	57.1	57.1	57.1	57.1
Zn rate (ppm)	0.0	0.0	0.0	0.0	60	60	60	60	0.0	0.0	0.0	0.0	60	60	60	60
P_2O_5 (ppm)	0.0	600	1200	1800	0.0	600	1200	1800	0.0	600	1200	1800	0.0	600	1200	1800

The application was carried out between 09.00 and 11.00 h. A summary of all treatments is shown in Table 2.

A randomized complete block design with four replications was used. Seeds were planted on the 3rd and 20th of April in seasons I and II, respectively, in plots having a size of 1.95 m x 4.0 m., including three ridges (after the precaution of border effect was taken into consideration). Hills were spaced 25 cm apart on one side of the ridge and seedlings were thinned to two plants/hill 6 weeks after planting, providing plant density of 123,000 plants/ha. Total irrigation amount during the growing season (surface irrigation) was about 6,000 m^3/ha. The first irrigation (after sowing irrigation) was given 3 weeks after sowing and the second was 3 weeks later. Thereafter, the plots were irrigated every 2 weeks until the end of the season, providing a total of nine irrigations. On the basis of soil test results, phosphorus fertilizer was applied at the rate of 54 kg P_2O_5 ha^{-1} as calcium super phosphate during land preparation. Nitrogen fertilizer was applied at the rate of 144 kg N ha^{-1} as ammonium nitrate with lime at two equal doses; the first one was applied after thinning just before the second irrigation and the other one was applied before the third irrigation (the recommended level for semi-fertile soil). Standard cultural practices of the experimental station were used.

At harvest, total cotton yield/plot was determined. Following ginning, the cottonseed yields were determined in kg/ha, along with seed index weight in g/100 seeds. Laboratory tests were conducted on a 200-g random sample of seed representative of each plot. A composite seed sample of the four replicates of each treatment was used for the chemical analyses. The following chemical analyses were conducted: (i) seed crude protein content according to AOAC [15]; (ii) seed oil content in which oil was extracted three times with chloroform/methanol (2:1, vol/vol) mixture according to the method outlined by Kates [16]; (iii) oil quality traits, i.e., refractive index, acid value, saponification value, unsaponifiable matter and iodine value were determined according to methods described by AOCS [17] and (iv) identification and determination of oil fatty acids by gas-liquid chromatography. The lipid materials were saponified, unsaponifiable matter was removed and the fatty acids were separated. The free fatty acids were methylated with diazomethane [18]. The fatty acid methyl esters were analyzed by a Hewlett Packard model 5890 gas chromatograph (Palo Alto, CA) equipped with dual flame-ionization detectors. The separation procedures were similar to those reported by Ashoub *et al*., [19] as follows: The chromatograph was fitted with an FFAP (cross-linked) 30 m (length) x 0.32 mm (column i.d.) x 0.25 µm (film thickness) capillary column coated with polyethylene glycol. The column oven temperature was programmed at 7°C/min from 50 to 240°C and kept finally to 30 min. Injector and detector temperatures were 250 and 260°C, respectively. Under these conditions, all peaks from C8 to 20 homologous series were well defined. Peak identification was performed by comparison of the relative retention time (RRT) for each peak with those of standard chromatograms. The RRT of oleic acid was given a value of 1.0. Results were expressed as an area percentage of chromatograms.

Statistical analysis. Data obtained for the cottonseed yield and seed index were statistically analyzed as a factorial experiment in a randomized complete blocks design following the procedure outlined by Snedecor and Cochran [20] and the least significant difference (LSD) was used to determine the significance of differences between treatment means at 0.05 level. As for the chemical properties considered in the study, the t-test computed in accordance with standard deviation was utilized to verify the significance between every two-treatment means at the 0.05 level of significance.

RESULTS AND DISCUSSION

Cottonseed yield: Cottonseed yield per hectare significantly increased when K was applied (by as much as 13.99%) (Table 3). Potassium would have a favorable impact on yield components, including number of opened bolls/plant and boll weight, leading to a higher cotton yield. The role of K suggests that it affects abscission (reduced boll shedding) and it certainly affects yield [21]. Pettigrew [1] indicated that, the elevated carbohydrate concentrations remaining in source tissue, such as leaves, appear to be part of the overall effect of K deficiency in reducing the amount of photosynthate available for reproductive sinks and thereby producing changes in boll weight. Furthermore, K has an important role in the

Table 3: Effect of K rate and foliar application of Zn and P on cottonseed yield, seed index, seed oil, seed protein, oil and protein yields

Treatments	Cottonseed yield (kg/ha)[a]	Seed index (g)[a]	Seed oil (%)[b]	Oil yield (kg/ha)[b]	Seed protein (%)[b]	Protein yield (kg/ha)[b]
K_2O-rate (kg/ha)						
0, control	1828.0	10.01	19.55	357.5	22.24	406.6
57.1	2083.8[d]	10.16[d]	19.82[d]	413.2[d]	22.27	464.1[d]
LSD 0.05[c]	80.61	0.054	-	-	-	-
SD[c]	-	-	0.153	34.22	0.038	36.25
Zn-rate (ppm)						
0, control	1868.3	10.04	19.59	366.2	22.25	415.7
60	2043.5[d]	10.13[d]	19.78[d]	404.4	22.26	455.0
LSD 0.05[c]	80.61	0.054	-	-	-	-
SD[c]	-	-	0.184	40.5	0.040	42.62
P_2O_5-rate (ppm)						
0, control	1775.8	9.97	19.56	347.5	22.23	394.8
600	1944.3[d]	10.08[d]	19.64	382.1	22.25	432.7
1200	2023.7[d]	10.13[d]	19.76	400.3[d]	22.26	450.5[d]
1800	2079.8[d]	10.16[d]	19.77	411.5[d]	22.28	463.3[d]
LSD 0.05[c]	114.01	0.077	-	-	-	-
SD[c]	-	-	0.202	40.21	0.040	41.78

[a]Combined statistical analysis from the two seasons, [b]Mean data from a four replicate composites for the two seasons, [c]LSD = Least significant differences, SD = Standard deviation was used to conduct t-test to verify the significance between every two treatment means at 0.05 level, [d]Significant at 0.05 level

translocation of photosynthates from sources to reproductive sinks [6]. Gormus [22] found that K application increased yield.

Application of Zn significantly increased cottonseed yield per hectare, as compared with the untreated control (by 9.38%). Possible explanation of such results might be due the improvement of yield components due to the application of Zn. Zinc could have a favourable effect on photosynthetic activity of leaves [23], which improves mobilization of photosynthates and directly influences boll weight. Further, Zn is required in the synthesis of tryptophan, a precursor of indole-3-acetic acid [7], which is the major hormone inhibits abscission of squares and bolls. Thus the number of retained bolls/plant and consequently cottonseed yield per hectare, would be increased [24].

Phosphorus application at all the three concentrations (600, 1200 and 1800 ppm as P_2O_5) also significantly increased cottonseed yield per hectare, where the three concentrations applied proved to excel the control (by 9.49-17.12%). Best yield was obtained at the high P_2O_5-concentration. Such results reflect the pronounced improvement of yield components due to application of P_2O_5 which is possibly ascribed to its involvement in photosynthesis and translocation of carbohydrates to young bolls [10, 12]. Phosphorus as a constituent of cell nucleus, is essential for cell division and development of meristematic tissue and hence it would have a stimulating effect on increasing the number of flowers and bolls per plant [11]. This result agreed with that reported by Katkar *et al*., [25].

Seed index: Seed index significantly increased with applying K (Table 3). Possible explanation for increased seed index due to the application of K may be due in part to its favorable effects on photosynthetic activity rate of crop leaves and CO_2 assimilation [5], which improves mobilization of photosynthates and directly influences boll weight that directly affect seed weight [26].

Application of Zn significantly increased seed index, as compared to control. Possible explanation for the increased seed weight might be due to increased photosynthesis activity resulting from the application of Zn [23], which improves mobilization of photosynthates and the amount of photosynthate available for reproductive sinks and thereby influences boll weight, factors that coincide with increased in seed weight [24].

Phosphorus applied at all the three rates significantly increased seed index over the control. The highest rate of P_2O_5 (1800 ppm) showed the highest numerical value of seed index. Possible explanation for increased seed weight due to the application of P is that this nutrient activated the biological reaction in cotton plant, particularly photosynthesis fixation of CO_2 and synthesis of sugar and other organic compounds [3]. This indicates that treated cotton bolls had larger photosynthetically supplied sinks for carbohydrates and other metabolites than untreated bolls.

Seed oil content and yield: Applied K caused significant increase in seed oil content and oil yield per hectare (55.7 kg oil/ha), compared with untreated control (Table 3). This could be attributed to the role of K in

Table 4: Effect of K rate and foliar application of Zn and P on seed oil properties[a]

Treatments	Refractive index	Acid value	Saponification value	Unsaponifiable matter (%)	Iodine value
K_2O-rate (kg/ha)					
0, control	1.4684	0.1343	190.81	0.3538	127.48
57.1	1.4698[c]	0.1316	189.74[c]	0.3950[c]	132.76[c]
SD[b]	0.00136	0.00322	0.742	0.02234	3.633
Zn-rate (ppm)					
0, control	1.4683	0.1336	190.71	0.3625	128.39
60	1.4699[c]	0.1323	189.84[c]	0.3863	131.85
SD[b]	0.00129	0.00346	0.809	0.02870	4.211
P_2O_5-rate (ppm)					
0, control	1.4681	0.1350	190.75	0.3525	125.33
600	1.4693	0.1343	190.33	0.3725	131.46[c]
1200	1.4696	0.1323	190.10	0.3800	131.93[c]
1800	1.4695	0.1309	189.92	0.3925	131.76[c]
SD[b]	0.00152	0.00339	0.944	0.02947	3.801

[a]Mean data from a four replicate composites for the two seasons, [b]SD = Standard deviation, [c]Significant at 0.05 level

biochemical pathways in plants. Potassium increases the photosynthetic rates of crop leaves, CO_2 assimilation and facilitates carbon movement [5]. Favorable effects of K on seed oil content and oil yield were mentioned by Abou El-Nour *et al*., [27].

Spraying plants with Zn resulted in an increase in seed oil content and oil yield per hectare (38.2 kg oil/ha), compared with untreated control. Cakmak [28] has speculated that Zn deficiency stress may inhibit the activities of a number of antioxidant enzymes, resulting in extensive oxidative damage to membrane lipids. Similar results were reached by Rathinavel *et al*., [24].

Application of P at all the three concentrations tended to increase the seed oil content and oil yield per hectare (34.6-64.0 kg oil/ha), over the control. The effect was significant due to the high P concentration (1800 ppm P_2O_5) on oil yield per hectare. This may be attributed to that P is required for production of high quality seeds, since it occurs as coenzymes involved in energy transfer reactions; energy is tapped in photosynthesis in form of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADP). This energy is then used in photosynthetic fixation of CO_2 and the synthesis of lipids and other essential organic compounds [3]. These results agreed with those obtained by Rajendran and Veeraputhiran [29], in sunflower.

Seed protein content and yield: Applied K caused slight increase in seed protein content and significantly increased protein yield per hectare (57.5 kg protein/ha), compared with untreated control (Table 3). It also, increased the protein yield per hectare, resulting in an improvement in both cottonseed yield and seed protein content. This could be attributed to the role of K in biochemical pathways in plants. Potassium increases the photosynthetic rates of crop leaves, CO_2 assimilation and facilitates carbon movement [5]. Also, K has favorable effects on metabolism of nucleic acids and proteins [12, 30]. These are manifested in metabolites formed in plant tissues and directly influence the growth and development processes. Similar results were obtained Abou El-Nour *et al*., [27] and Ghourab *et al*., [26].

Application of Zn slightly increased the seed protein content and increased protein yield per hectare (39.3 kg protein/ha) numerically, compared with the untreated control. Because Zn is directly involved in both gene expression and protein synthesis, Cakmak [28] has speculated that Zn deficiency stress may inhibit the activities of a number of antioxidant enzymes, resulting in extensive oxidative damage to proteins, chlorophyll and nucleic acids. These results agreed with those reported by Babhulkar *et al*., [31], in safflower.

Phosphorus applied at all rates tended to increase the seed protein content and the protein yield per hectare (37.9-68.5 kg protein/ha), compared with untreated control. The effect was significant when applied the high P concentration (1800 ppm P_2O_5) on protein yield per hectare, resulting from an improvement in both cottonseed yield and seed protein content. Best protein yield was obtained at the high P_2O_5-concentration. Phosphorus is a component of nucleic acids, which are necessary for protein synthesis [3]. Similar results were obtained by Tomar *et al*., [32], in sunflower.

Seed oil properties: The oil refractive index, unsaponifiable matter and iodine value, tended to increase, while the acid value and saponification value tended to decrease by applied K, compared with the untreated control (Table 4). A significant effect was found concerning, saponification value, unsaponifiable matter and iodine value. The increment of the

Table 5: Effect of K rate and foliar application of Zn and P on the relative percentage of saturated fatty acids[a]

	Relative % of saturated fatty acids					
Treatments	Capric	Lauric	Myristic	Palmitic	Stearic	Total
K_2O-rate (kg/ha)						
0, control	0.0774	0.0626	0.8275	22.21	2.271	25.4525
57.1	0.0728[c]	0.0599	0.4863[c]	19.72[c]	1.915	22.2501[c]
SD[b]	0.00369	0.00794	0.34079	1.482	0.4512	2.33093
Zn-rate (ppm)						
0, control	0.0769	0.0609	0.6763	22.16	2.185	25.1590
60	0.0733	0.0616	0.6375	19.77[c]	2.001	22.5436[c]
SD[b]	0.00400	0.00496	0.38598	1.796	0.4798	2.53159
P_2O_5-rate (ppm)						
0, control	0.0795	0.0665	1.1075	22.80	2.728	26.7760
600	0.0748[c]	0.0623[c]	0.5925[c]	20.70	1.855[c]	23.2870[c]
1200	0.0733[c]	0.0595[c]	0.4375[c]	20.30	1.905[c]	22.7703[c]
1800	0.0728[c]	0.0568[c]	0.4900[c]	20.07	1.885[c]	22.5720[c]
SD[b]	0.00368	0.00340	0.28269	2.026	0.3173	2.42171

[a]Mean data from a four replicate composites for the two seasons, [b]SD = Standard deviation, [c]Significant at 0.05 level

Table 6: Effect of K rate and foliar application of Zn and P on the relative percentage of unsaturated fatty acids[a]

	Relative % of unsaturated fatty acids			
Treatments	Oleic	Linoleic	Total	TU/TS[b] ratio
K_2O-rate (kg/ha)				
0, control	21.61	52.94	74.54	2.954
57.1	22.73	55.01[d]	77.75[d]	3.538[d]
SD[c]	1.407	1.498	2.332	0.4037
Zn-rate (ppm)				
0, control	21.43	53.40	74.84	3.016
60	22.90[d]	54.55	77.45[d]	3.476[d]
SD[c]	1.311	1.761	2.533	0.4469
P_2O_5-rate (ppm)				
0, control	21.11	52.11	73.22	2.755
600	21.96	54.75[d]	76.71[d]	3.331[d]
1200	22.52	54.70[d]	77.23[d]	3.427[d]
1800	23.09[d]	54.33[d]	77.43[d]	3.472[d]
SD[c]	1.421	1.571	2.422	0.4392

[a]Mean data from a four replicate composite for the two seasons,
[b]TU/TS ratio = (total unsaturated fatty acids) / (total saturated fatty acids),
[c]SD = Standard deviation, [d]Significant at 0.05 level

unsaponifiable matter is known to be beneficial for its role in oil stability, thus its components need further study. Potassium is an essential nutrient and an integral component of several important compounds in plant cells. This attributed to the role of K in biochemical pathways in plants [4]. These may be reflected in distinct changes in seed oil quality. Mekki *et al*., [33] stated that, foliar application with K (0 or 3.5% K_2O) on sunflower at the seed-filling stage resulted in decreased oil acid content. Froment *et al*., [34] in linseed, found that the iodine value, which indicates the degree of unsaturation in the final oil, was highest in treatments receiving extra K.

Spraying plants with Zn resulted in an increase in oil refractive index, unsaponifiable matter and iodine value and a decrease in acid value and saponification value, compared with untreated control. Zn activates a large number of enzymes, either due to binding enzymes and substrates, or effects of Zn on conformation of enzymes or substrate, or both [25, 36], these would have direct impact through utilization in growth processes, which are reflected in distinct changes in seed oil quality.

Application of P at different concentrations tended to decrease the oil acid value and saponification value, while tended to increase the unsaponifiable matter and iodine value especially when applied the high P concentration (1800 ppm P_2O_5), compared with the untreated control. Significant results were obtained when applied P at all concentrations on iodine value. The effect of P_2O_5-concentrations on oil refractive index was very limited with no definite trend. The studied oil quality characters seemed to be enzymatically controlled.

Oil fatty acids composition: Applied K decreased the oil-saturated fatty acids (capric, lauric, myristic, palmitic and stearic) (Table 5). A significant effect was found only on capric, palmitic and the total saturated fatty acids. The total unsaturated fatty acids (oleic and linoleic) and the ratio between total unsaturated fatty acids and total saturated fatty acids (TU/TS) were increased (by 4.31 and 19.77%, respectively) by applied K (Table 6). The effect was significant on linoleic acid, the total unsaturated fatty acids (oleic and linoleic) and the ratio between total unsaturated fatty acids and total saturated fatty acids (TU/TS). The beneficial effect of applied K on TU and TU/TS ratio may be due to the regulated effect of K, which acts as an activator on many enzymic processes, where some of these enzymes may affect the seed oil content from these organic matters. To our knowledge no information on the effect of K on the cottonseed oil fatty acids was found in the available literatures. Mekki *et al*., [33] stated that, foliar application with K on sunflower increased the oleic acid fatty acid.

Froment *et al*., [34], in linseed oil, found that linoleic acid content was greatest in treatment receiving extra K.

Application of Zn resulted in a decrease of the saturated fatty acids, i.e. palmitic, capric, myristic and stearic and the total ones, while resulted in an increase in lauric acid, compared to untreated control. The effect was significant only on palmitic acid and the total saturated fatty acids oil. Application of Zn resulted in an increase in total unsaturated fatty acids (by 3.49%) and TU/TS ratio (by 15.25%), over the control. The effect was significant on oleic acid, the total unsaturated fatty acids (oleic and linoleic) and the ratio between total unsaturated fatty acids and total saturated fatty acids (TU/TS). The stimulatory residual effects of application Zn on TU and TU/TS ratio was probably due to its favorable effects on fundamental metabolic reactions in plant tissues [2]. Also, a large number of enzymes are activated by Zn, either due to binding enzymes and substrates, or effects of Zn on conformation of enzymes or substrate, or both [35, 36]. Further, a key role of Zn in gene expression and regulation has been reported by Klug and Rhodes [35]. Some of these effects for Zn may affect the seed oil fatty acids composition.

Phosphorus applied at all concentrations resulted in a decrease in the total saturated fatty acids compared with untreated control. Spraying plants with P_2O_5 at 1800 ppm gave the lowest total saturated fatty acids oil, followed by 1200 ppm concentration, compared with the control. Applied the high P concentration (1800 ppm P_2O_5) gave the lowest capric, lauric, palmitic and stearic acids contents, compared with the other two concentrations (600 and 1200 ppm), while applied P_2O_5 at 1200 ppm gave the lowest myristic acid content, compared with the other two concentrations (600 and 1800 ppm). The effect was significant for the two concentrations 1200 and 1800 ppm P_2O_5 on capric acid and the total saturated fatty acids oil and for all different P concentrations on lauric, myristic and stearic. Phosphorus applied at all rates increased the total unsaturated fatty acid (by 4.77-5.75%) and TU/TS ratio (by 20.91-26.03%), compared with untreated control. Applied P_2O_5 at 1800 ppm gave the highest increment, followed by 1200 ppm concentration. Spraying plants with P_2O_5 at 1800 ppm produced seed oil characterized by highest oleic acid content, while spraying with 600 ppm gave the highest linoleic acid content, compared with the other concentrations. The effect was significant for the high P concentration (1800 ppm P_2O_5) on oleic, for the two concentrations 1200 and 1800 ppm P_2O_5 on the TU/TS ratio and for all different concentrations on linoleic and total unsaturated fatty acid. The beneficial effect of applied P at different concentrations on TU and TU/TS ratio may be due to the regulated effect of P on many enzymic processes and P also acts as an activator of some enzymes [37], which may affect the seed oil fatty acids composition. Gushevilov and Palaveeva [38] studied the changes in sunflower oil contents of linoleic, oleic, stearic and palmitic acids due to P-application rate and found that oil quality remained high at high P-rate. Khan *et al*., [39] indicated that, oleic acid increased by increasing levels of P added to rapeseed-mustard.

Low content of saturated fatty acids is desirable for edible uses. Also, regarding oil quality, higher levels of linoleic acid and oleic acid are considered good for oil quality [40].

During the two growing seasons no significant interactions were found between the variables in the present study (application of K, Zn and P_2O_5 concentrations) on quantitative and qualitative characters under investigation. Regarding insignificant interaction effects, the F ratios worthy exceed unity, but within the level of probability take p#0.05, they did not show significance.

CONCLUSIONS

From the findings of the present study, it seems rational to recommend addition of K at 57.1 kg K_2O/ha, spraying cotton plants with Zn twice (at 60 ppm) and application of P_2O_5, also twice (especially the P_2O_5-concentration of 1800 ppm). These combinations appeared to be the most beneficial treatments, affecting not only the quantity but also the quality of oil and to obtain higher oil, protein yields and a better fatty acid profile in the oil of cotton. In comparison with the ordinary cultural practices adopted by Egyptian cotton producers, it is quite apparent that applications of such treatments could bring about better impact on cottonseed yield, seed protein content, oil and protein yields, oil refractive index, unsaponifiable matter, iodine value and unsaturated fatty acids and a decrease in oil acid value and saponification value. The increase in seed yield and subsequent increase in oil and meal due to the addition of K, spraying cotton plants with Zn and of P_2O_5 are believed to be sufficient enough to cover the cost of using those chemicals and to attain an economical profit.

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Physiological Effect of Diphenylamin and Tryptophan on the Growth and Chemical Constituents of *Philodendron erubescens* Plants

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Abstract: A pot experiment was carried out in the nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during the two successive seasons of 2003/2004 and 2004/2005, with the aim of studying the effect of foliar application of the amino acids diphenylamin and tryptophan (each at the rates of 50 or 100 ppm), applied separately or in combinations of the different concentrations (plus untreated control plants), on the growth and chemical constituents of *Philodendron erubescens* plants. The most important results can be summarized as: 1) Both diphenylamin and tryptophan significantly increased plant growth (in terms of plant height, number of leaves/plant, stem diameter, root length, leaf area, as well as fresh and dry weights of the different plant parts) and the contents of carotenoids, total soluble sugars and total free amino acids in the leaves. 2) The effect of diphenylamin was superior to that of tryptophan on increasing plant growth and chemical constituents. 3) In both seasons, the maximum plant growth (as determined by all the recorded parameters) was obtained from plants treated with diphenylamin at the rate of 100 ppm, followed by tryptophan at 100 ppm. 4) Amino acids treatments had no significant effect on the chlorophyll A and B contents in both seasons. However, the total chlorophyll content was significantly increased in the second seasons as a result of the amino acid treatments. 5) Plant treated with diphenylamin at 100 ppm had the highest contents of caroteniodes, total soluble sugars and total free amino acids in the leaves.

Key words: Diphenylamin % Tryptophan % *Philodendron erubescens* % amino acids

INTRODUCTION

The regulation of plant growth and biosynthesis of important economic chemical constituents could be achieved through the use of different growth regulating substances. There has been a recent trend to use naturally-occurring compounds (including amino acids) to achieve such regulation. Davies [1] reported that amino acids as organic nitrogenous compounds are the building blocks in the synthesis of proteins, which are formed by a process in which ribosomes catalyze the polymerization of amino acids.

Several hypotheses have been proposed to explain the role of amino acids in plant growth. Available evidence suggests several alternative routes of IAA synthesis in plants, all starting from amino acids [2]. On the other hand, Waller and Nowaki [3] suggested that the regulatory effects of certain amino acids, like phenylalanine and ornithine, on plant development is through their influence on gibberellins.

The role of tryptophan is well known: it has an indirect role on the growth via its influence on auxin synthesis. Phillips [4] reported that alternative routes of IAA synthesis exist in plants, all starting from tryptophan. Thus, when tryptophan was supplied to some plant tissues IAA was formed. Moursy *et al.,* [5] established callus lines of *Datura stramonium* L. and found that phenylalanine and ornithine increased both fresh and dry weights of callus compared with the control. Moreover, there have been reports that foliar application of amino acids (Lysine, ornithine, salicylic acid and tryptophan) enhanced the vegetative growth and chemical constituents [Talaat and Youssef [6] on basil plants, Talaat [7] on *Pelargonium graveolens* L. and Talaat *et al.,* [8] on *Catharanthus roseus* L.].

Philodendron erubescens 'Red Emerald' plants (Family: Araceae) is one of the most important house plants. It has waxy arrow-shaped leaves, up to 25 cm in length, bronzy green in colour, with red edges and wine-red beneath. The petioles are green with red and are occasionally winged.

The aim of this work was to study the effect of the amino acids diphenylamine and tryptophan, as well as and their combinations, on growth and chemical

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constituents of *Philodendron erubescens* plants and the feasibility of using these chemicals to improve plant quality.

MATERIALS AND METHODS

A pot experiment was carried out during two seasons (2003/2004 and 2004/2005) at the nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, with the aim of studying the effect of foliar applications of the amino acids diphenylamin and tryptophan on the growth and chemical constituents of *Philodendron erubescens* 'Red Emerald' plants.

Plant material: Plantlets of *Philodendron erubescens* (6 months old, with 2 leaves/plantlet and an average height of 13-15 cm) were transplanted in pots (16 cm in diameter) filled with a mixture of sand and peat moss (1:1, v/v) on September 20th, 2003 and 2004, in the first and second seasons, respectively.

The seedlings were placed in an uncontrolled glasshouse. Each pot was supplied with 2 g of an NPK (15:15:15) fertilizer. The fertilizers were repeated with a total of 6 applications, at one-month intervals, starting after 21 days from transplanting. Other common cultural practices were preformed as needed. The plants were sprayed with the amino acids diphenylamin and tryptophan, (each at concentrations of 50 or 100 ppm) applied either separately, or in combinations (at concentrations of 50:50, 50:100, 100:50 or 100:100 ppm of diphenylamin and tryptophan, respectively), in addition to the untreated control plants. The amino acids foliar spray treatments were applied one month after transplanting (on October 20th in both seasons) and were repeated 4 times at one-month intervals.

The pots were arranged in a randomized complete blocks design, with 9 treatments (control plus eight amino acid treatments), replicated three times, with each replicate (block) consisting of 10 plants/treatment.

At the termination of the experiment (on April 20th in both seasons), the following data were recorded:

Measurements of growth parameters:

- Plant height (cm).
- Number of leaves/plant.
- Stem diameter (mm) at a height of 5 cm from soil surface.
- Root length (cm).
- Leaf area (cm^2) of 4th leaf from soil surface.
- Fresh and dry weights of leaves, stems and roots (g/plant).

Chemical analysis:

- Fresh leaf samples were collected from plants receiving the different treatments and were chemically analyzed to determine their content of photosynthetic pigments (chlorophyll A, chlorophyll B and carotenoids) using the method described by Von Wettstein [9].
- Leaf samples were dried and their contents of total soluble sugars were determined according to Dubois *et al.,* [10].
- The content of total free amino acids in leaves was determined according to Rosein [11].

Statistical analysis: Data obtained were subjected to analysis of variance and the means were compared using the Duncan's Multiple Range Test (at the 0.05 level), as recommended by Snedecor and Cochran [12].

RESULTS AND DISCUSSION

Effect of amino acids on plant growth:

Plant height (cm): Treatment of *Philodendron erubescens* plants with the amino acids diphenylamin and tryptophan had a significant effect on plant height (Table 1). In the first season, the plant height ranged from 25.60 to 46.30 cm, whereas in the second season, the recorded values ranged from 27.30 cm to 47.30 cm. In both seasons, the shortest plants were those that were untreated (control). Treatment of the plants with any of the tested amino acid treatments caused a significant increase in plant height and the percentages of increases (compared to the control plants) ranged from 31.25 to 80.86% in the first season and from 12.09 to 73.26% in the second season. The highest percentage of increase was found in plants which were treated by diphenylamin at 100 ppm, which gave the highest values in both seasons (46.30 and 47.30 cm in the first and second seasons, respectively).

The comparison between diphenylamin and tryptophan revealed the superior effect of diphenylamine, especially at the rate of 100 ppm, over tryptophan in the two seasons.

The role of the amino acids in stimulating growth of several plant species was studied by Phillips [4], who indicated that several alternative routes of IAA synthesis exist in plants, all starting from amino acids. Russell [13] reported that the increase in growth as a result of

Table 1: Effect of diphenylamin (Dip) and tryptophan (Try) on growth of *Philodendron erubescens* plants

Treatments	Plant height (cm)	Number of leaves/plant	Stem diameter (mm)	Root length (cm)	Leaf area (cm^2)
	First season (2003/2004)				
Control	25.60F	7.00E	7.60F	14.30F	913.0G
Dip.50 ppm	44.00B	10.60BC	9.20B	19.30E	1324.0C
Dip.100 ppm	46.30A	13.30A	10.30A	35.00A	1765.0A
Try.50 ppm	37.60D	10.00B-D	9.10B	19.30E	1232.0DE
Try.100 ppm	37.60D	11.00B	10.20A	24.30D	1223.0E
Dip+Try (50:50 ppm)	44.30B	9.00CD	8.30D	19.60E	936.0G
Dip+Try (50:100 ppm)	39.00C	10.00B-D	8.60C	19.00E	1386.0B
Dip+Try (100:50 ppm)	38.00CD	8.90DE	8.10E	32.30B	1281.0CD
Dip+Try (100:100 ppm)	33.60E	10.00B-D	8.60C	30.60C	1119.0F
LSD 0.5%	1.249	1.683	0.172	1.521	50.220
	Second season (2004/2005)				
Control	27.30G	8.00F	7.90I	15.60G	943.0G
Dip.50 ppm	45.60B	11.30CD	10.30C	21.30E	1369.0C
Dip.100 ppm	47.30A	14.60A	11.00A	36.10A	1820.0A
Try.50 ppm	40.30D	11.00D	10.00D	20.60E	1330.0CD
Try.100 ppm	36.60E	13.30B	10.90B	26.60D	1320.0CD
Dip+Try (50:50)	46.60AB	10.60D	9.30F	18.30F	1001.0F
Dip+Try (50:100)	41.60C	12.30BC	9.60E	17.60F	1405.0B
Dip+Try (100:50)	39.60D	9.30E	8.60H	34.60B	1311.0D
Dip+Try(100:100)	30.60F	11.60CD	9.00G	31.60C	1190.0E
LSD 0.5%	1.108	1.241	0.079	1.239	49.59

Within each column, values followed by the same alphabetical letter(s) are not significantly different according to Duncan's multiple range test (at the 0.05 level)

application of amino acids may be due to their conversion into IAA. Attoa *et al.*, [14] reported that spraying *Iberis amara* L. plants with the amino acid tryptophan increased plant growth. Regarding the effect of amino acids on plant height, the results of this study are in agreement with those obtained by Salonen [15] on *Atropa belladomna*, Moursy *et al.*, [5] on *Datura stramonium*, El-Bahar *et al.*, [16] on *Datura metel*, Gamal El-Din [17] on *Hyoscyamus muticus* and Talaat and Youssef [6] on *Ocimum basilicum*, who reported that foliar applications of amino acids significantly promoted plant growth.

In conclusion, it can be stated that diphenylamin (100 ppm) was the most effective treatment in causing a significant increase in the length of *Philodendron* plants.

Number of leaves/plant: The data recorded in this study (Table 1) indicated that the effect of the different treatments on the number of leaves/plant was generally similar to their effect on plant height. In both seasons, all the diphenylamin or tryptophan treatments that were used in this study significantly increased the number of leaves/plant, compared to the control. The percentage of increase recorded with the different treatments varied from 27.14 to 90.00% in the first season and from 16.25 to 82.50% in the second season, over the untreated plants. The data in Table 1 also show that the effect of diphenylamin (especially at the rate of 100 ppm) on the number of leaves/plant was superior to that of tryptophan. In fact, the highest percentage of increase in both seasons was recorded on plants that were treated with diphenylamin at 100 ppm. This means that this treatment was the most effective one for causing the greatest significant increase in the production of leaves by the plants (i.e. giving the highest number of leaves/plant), compared to the other treatments. In contrast, the shortest plants were those that received no treatment (control).

From the above results, it can be concluded that the amino acids diphenylamin and tryptophan had a stimulating effect on the production of leaves (i.e. they increased the number of leaves/plant), with diphenylamine (especially at 100 ppm) being more effective in this respect than tryptophan. The number of leaves/plants is one of the most important criteria in the foliage plants, by which plant quality is determined.

Stem diameter (mm): The data (Table 1) revealed that the response of stem diameter to the amino acid treatments followed the same trend as in plant height and number of leaves/plant. In both seasons, plants receiving the different treatments had significantly thicker stems than the untreated control plants. Here also, the application of diphenylamin at the rate of 100 ppm was the most effective treatment in increasing stem thickness, giving the greatest mean stem diameters in both seasons (10.30 and 11.0 mm in the first and second seasons,

Table 2: Effect of diphenylamin (Dip) and tryptophan (Try) on the fresh and dry weights (g/plant) of different parts of *Philodendron erubescens* plants

	Fresh weight (g/plant)				Dry weight (g/plant)			
Treatments	Leaves	Stems	Roots	Total	Leaves	Stems	Roots	Total
	First season (2003/2004)							
Control	25.4F	21.8G	7.4E	54.6G	3.5C	2.9G	2.2F	8.6F
Dip.50 ppm	44.7C	36.2C	8.7E	89.6D	5.5AB	4.3CE	2.6EF	12.4CD
Dip.100 ppm	66.8A	51.8A	16.3A	134.0A	7.2A	5.7A	4.9A	17.8A
Try.50 ppm	46.7B	36.7C	10.4D	93.8C	4.8BC	4.0DF	2.6EF	11.4DE
Try.100 ppm	45.1C	38.4B	12.6BC	96.1B	6.2AB	5.3AB	2.9DE	14.4B
Dip+Try (50:50)	36.6E	28.9F	10.7D	76.2F	4.6BC	3.4FG	2.4EF	9.9EF
Dip+Try (50:100)	40.7D	33.9E	11.7CD	86.3E	5.7AB	4.5BD	3.6C	13.8BC
Dip+Try (100:50)	45.5BC	38.1B	13.4B	97.0B	5.9AB	5.0AC	3.4CD	14.3B
Dip+Try (100:100)	45.7BC	35.0D	15.4A	96.1B	5.0BC	3.6FG	4.4B	13.0BC
LSD 0.5%	1.521	0.538	1.472	1.600	1.717	0.832	0.576	1.608
	Second season (2004/2005)							
Control	22.9H	26.4E	9.2F	58.5H	3.5C	4.2D	3.0B	10.7F
Dip.50 ppm	38.1DE	34.1D	10.1EF	82.3F	5.1AB	6.2BC	3.1B	14.4DE
Dip.100 ppm	53.8A	69.9A	19.2B	142.9A	6.2A	8.1A	5.2A	19.5A
Try.50 ppm	38.7CD	48.7B	11.6DE	99.0D	4.9AC	5.3B	3.1B	13.3E
Try.100 ppm	40.3B	47.3B	13.9C	101.5C	6.1A	6.9BC	3.8B	16.8B
Dip+Try (50:50)	31.3G	35.1D	12.1D	78.5G	4.2BC	5.8BC	3.3B	13.3E
Dip+Try (50:100)	35.0F	42.9C	13.9C	91.8E	5.2AB	6.2BC	4.4B	15.8BC
Dip+Try (100:50)	40.0BC	47.5B	14.9C	102.4C	5.9A	6.4BC	4.2AB	16.5B
Dip+Try (100:100)	36.7E	48.1B	16.9B	101.7B	4.1BC	5.9BC	5.1A	15.1CD
LSD 0.5%	1.588	2.335	1.588	1.087	1.549	1.126	1.548	1.083

Within each column, values followed by the same alphabetical letter(s) are not significantly different according to Duncan's multiple range test (at the 0.05 level)

respectively). These values reflect increases of 35.5 and 39.2% in stem diameter in the first and second season, respectively, compared to the control.

Root length (cm): The data (Table 1) showed that the effect of the amino acid treatments on root length followed the same trend as that detected for plant height, number of leaves/plant and stem diameter. Using diphenylamin at 100 ppm gave significantly longer roots than those of plants receiving any other treatment. The percentages of increase in root length as a result of this treatment (over the control) reached 144.8% and 131.4% in first and second seasons, respectively.

It has been reported that the amino acid lysine is converted into the diamine cadaverine by decarbotylation. Its synthesis is catalyzed by lysine decarboxylase. Cadaverine may play an important role in root development [18].

Leaf area (cm^2): The data presented in Table 1 showed that treatment of *Philodendron erubescens* plants with the amino acids diphenylamin and tryptophan significantly increased the leaf area. In both seasons, the smallest leaves (913 and 943 cm^2 in the first and second seasons, respectively) were those of the untreated plants. On the other hand, the largest leaves (1765 and 1820 cm^2 in the two seasons, respectively) were found on plants that had been treated with diphenylamin at the concentration of 100 ppm. The percentages of increase in this character as a result of applying diphenylamin at 100 ppm (compared to the control) reached 93.3 and 93.0% in the first and second seasons, respectively. Similar increases in root length as a result of amino acid treatments have been previously observed by several investigators on a number of plant species Gamal El-Din *et al.,* [19] on lemon-grass (*Cymbopogon citratus*) and Talaat and Youssef [6] on *Ocimum basilicum*.

Fresh and dry weights of different plant parts (gm/plant): The data recorded in the two seasons (Table 2) showed that the amino acids diphenylamin and tryptophan significantly increased the fresh and dry weights of the different parts of the plant (leaves, stem, roots and the whole plants), compared to those of the untreated control plants. The most effective treatment was the application of diphenylamin at 100 ppm (in both seasons). This may be attributed to the fact that this treatment gave the longest plants, the greatest number of leaves/plants, the thickest stem diameter and the largest leaf area.

Comparing the effects of diphenylamin and tryptophan on the fresh and dry weights of the different plant parts, one can notice that diphenylamin was generally more effective than tryptophan.

Table 3: Effect of diphenylamin (Dip) and tryptophan (Try) on chemical constituents of *Philodendron erubescens* plants

Treatments	Chlorophyll A (mg/100 g FW)	Chlorophyll B (mg/100 g FW)	Total Chl. A+B (mg/100 g FW)	Caroteniodes (mg/g FW)	Total soluble sugars (mg/100 g DW)	Total free amino acids (mg/g DW)
	First season (2003/2004)					
Control	0.531A	0.257A	0.788A	1.039C	27.71F	8.45D
Dip.50 ppm	0.640A	0.296A	0.936A	1.068C	37.88AB	11.02C
Dip.100 ppm	1.118A	0.471A	1.588A	1.567A	41.17A	15.18A
Try.50 ppm	0.991A	0.369A	1.360A	1.407AB	33.59CD	13.53AB
Try.100 ppm	1.024A	0.451A	1.475A	1.531A	33.27CE	14.16A
Dip+Try (50:50)	1.098A	0.471A	1.569A	1.510A	31.85DE	13.60AB
Dip+Try (50:100)	0.970A	0.449A	1.419A	1.559A	35.65BC	11.76BC
Dip+Try (100:50)	0.911A	0.379A	1.290A	1.279B	30.24FE	11.33BC
Dip+Try (100:100)	0.999A	0.443A	1.442A	1.419AB	38.53AB	13.27AC
LSD 0.5%	0.798	0.612	0.802	1.660	3.303	2.343
	Second season (2004/2005)					
Control	0.542A	0.291A	0.833H	1.091B	29.25G	9.40D
Dip.50 ppm	0.649A	0.301A	0.950G	1.123B	39.90B	12.51C
Dip.100 ppm	1.201A	0.511A	1.712A	1.613A	44.20A	16.22A
Try.50 ppm	1.001A	0.391A	1.392F	1.501A	35.30D	14.67B
Try.100 ppm	1.113A	0.510A	1.623B	1.602A	34.90D	16.17A
Dip+Try (50:50)	1.007A	0.492A	1.499D	1.598A	30.51F	15.63A
Dip+Try (50:100)	0.989A	0.509A	1.498DE	1.610A	37.96C	12.98C
Dip+Try (100:50)	1.102A	0.391A	1.493E	1.495A	33.23E	12.21C
Dip+Try (100:100)	1.009A	0.481A	1.600C	1.502A	40.71B	14.33B
LSD 0.5%	0.804	0.614	0.0055	0.134	1.123	0.948

Within each column, values followed by the same alphabetical letter(s) are not significantly different according to Duncan's multiple range test (at the 0.05 level)

These results are in agreement with previous reports of Russell [13], Harridy [20] on *Catharanthus roseus*, Attoa *et al*., [14] on *Iberis amara* and Talaat *et al*., [8] on *Nigellia sativa*.

The increase in the fresh and dry weights as a result of the tryptophan treatments may be due to its conversion into IAA [13]. The percentages of increase in the total plant fresh weight due to spraying the plants with diphenylamin at 100 ppm (compared to the control) reached 145.2 and 111% in the first and second seasons, respectively. However, for the dry weight these percentages reached 104 and 80%, respectively.

Effect of amino acids on chemical constituents:

Photosynthetic pigments: The data presented in Table 3 showed that the amino acid treatments which were used in this study had no signifricant effect on the chlorophyll A, chlorophyll B and total chlorophyll contents in the first season, whereas in the second season, the foliar applications of all treatments caused a significant increase in the total chlorophyll (A+B) content, compared to that of control plants. Plants treated with 100 ppm diphenylamin had the highest total chlorophyll content (1.712 mg/100 g), while untreated plants gave the lowest value (0.833 mg/100 g).

The recorded data also revealed that plants treated with diphenylamin at 100 ppm had the highest carotenoides content, compared to that found in plants receiving any other treatment. The percentages of increase due to this treatment over the control reached 50.8 and 47.8% in the first and second seasons, respectively.

The comparison between the effects of diphenylamin and tryptophan revealed that the influence of diphenylamin on increasing the photosynthetic pigments (especially at the rate of 100 ppm, which can be considered as the most effective treatment) was superior to that of tryptophan.

The present data are in agreement with the findings of Milad [21] on *Mentha virids*, Shoala [22] on *Lavendula multifida* plants and Hassanein [23] on *Foeniculum vulgare*. They reported that foliar application of amino acids (tryptophan), caused an increase in the contents of photosynthetic pigments.

Total soluble sugars contents: Data in Table 3 indicated that application of the amino acids diphenylamin and tryptophan as a foliar spray caused a significant increase in the contents of total soluble sugars in the leaves. The most effective treatment in this respect was diphenylamin at 100 ppm, followed by diphenylamin plus tryptophan (at 100:100 ppm). Comparing the effects of diphenylamin and tryptophan, the data indicated that diphenylamin was superior to tryptophan. The percentages of increase in the

total soluble sugars content caused by treating the plants with diphenylamin at 100 ppm (compared to the control) were 48.6 and 51.1% in the first and second seasons, respectively.

These results are in agreements with those obtained by Talaat and Youssef [6] on *Ocimum basilicum* L. and Wahba *et al.*, [24] on *Antholyza aethiopica* plants.

Total free amino acids: The results recorded in the two seasons (Table 3) showed that spraying *Philodendron* plants with different amino acid treatments caused a significant increase in the content of total free amino acids in the leaves. In both seasons, application of the high diphenylamin concentration (100 ppm) was the most effective treatment in producing the highest values, followed by tryptophan at the rate of 100 ppm. The increase in the content of total free amino acids as a result of the tryptophan treatments may be attributed to its conversion of to IAA, as stated by Phillips [4].

The percentages of increase in total free amino acid contents as a result of using diphenylamin at 100 ppm (compared to the untreated plants) were 79.6 and 72.6% in the first and second seasons, respectively. Our results are in agreement with the findings of Harridy [20] on *Catharanthus roseus*.

In conclusion, it can be stated that treatment of *Philodendron erubescens* plants with diphenylamin (especially at the concentration of 100 ppm) or tryptophan had a beneficial effect plant growth and chemical constituents.

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Management of *Callosobruchus chinensis* Linnaeus Through Use of Resistance in Stored Chickpea Varieties

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Abstract: Six varieties of stored chickpea were tested for their resistance against *Callosobruchus chinensis* L. (CCL) in the Laboratory of Department of Entomology, University of Arid Agriculture Rawalpindi. It was concluded that Parbat proved to be highly susceptible against CCL as compared with Paidar-91, the susceptible standard. The variety CM-2000 proved to be susceptible.The variety Punjab-91 and Pb-2000 proved to be partially resistant while Bittle-98 proved to be resistant against CCL. There was a significant correlation among number of adults and number of eggs, number of adults and percent weight loss, number of adults and number of holes, number of eggs and weight loss, number of eggs and number of holes, percent weight loss and number of holes. Chemical analysis of different varieties showed variations in dry matter, moisture, crude protein, fat fiber, total mineral (ash) and tanin. The study shows that variety Bittle-98 is a promising one which can be incorporated in future management programmes against CCL.

Key words: Eggs % weight loss % number of holes % coefficient of correlation

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an ancient crop that has been grown in Pakistan India, the Middle East and parts of Africa for many years. The common names used for chickpea are Bengal gram (India), Garbanzo (Latin America), Hommes, Hamaz (Arab world), Nohud, Lablabi (Turkey), Shimbra (Ethiopia) and Chana in Pakistan. It has been grown in Turkey nearly 7400 years ago. Turkey is considered as the oldest cultivated land for this pulse crop. It provides high quality protein and considered to be best food for vegetarian population in India, South Asia, West Asia and Southern European countries [1]. Seeds have about 20% protein, 5% fat and 55% carbohydrate. Seeds are sold in markets either as dry or canned. Common uses in United States are in soups, vegetable combinations, or as a component of fresh salads in restaurant salad bars. Chickpea is valued for its nutritive seeds with high protein content, 25.3-28.9%, after dehulling [2]. These grain legumes are however, susceptible to different species of beetles of the family Bruchidae in storage commonly known as Dhora. The chickpea bruchid *Callosobruchus chinensis* L. (CCL) feeds on the chickpea and other peas. Based upon the percent infestation, CCL was declared as major pest of chickpea, as it caused more than 10% damage to chickpea [3]. These beetles bore into bean seed. The beans in case of severe infestation become completely hollow and are unmarketable but resistant varieties can tolerate the effects of CCL. The best plant protection should base on host plant resistance and technique is suitable for subsistence farming in dry regions [4]. Some studies on susceptible and resistant varieties were also done. Screening of chickpea varieties for oviposition, larval development, weight loss, number of holes was done by Jha [5]. The present research study was carried out to screen the resistant varieties of chickpea (*Cicer arietinum* L.) from germplasm for their incorporation in management programmes against CCL by studying the damaging behavior of CCL on different germplasm of *Cicer arietinum* Linnaeus.

MATERIALS AND METHODS

To check resistance of different genotypes of chickpea against CCL, six varieties namely CM-2000, Punjab-91, Parbat, Bittle-98, Pb-2000 and Paidar-91 were used. These varieties were collected from Pulse Section, National Agriculture Research Center, Islamabad. The experiment was performed under laboratory conditions in the Department of Entomology, University of Arid Agriculture, Rawalpindi. Varieties were fumigated by

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Table 1: Comparison of means

Varieties	No. of adults	No. of eggs	% weight loss	No. of holes
Parbat	7.23±0.76 c	5.19±0.67 c	8.21±0.57 d	1.43±0.02 c
Pb-2000	0.67±0.05 a	1.58±0.48 a	4.90±0.60 ab	0.22±0.16 a
Paidar-91	2.90±0.54 b	3.11±0.46 b	6.92±0.66 cd	1.03±0.17 b
CM-2000	1.10±0.43 a	1.23±0.05 a	6.21±0.55 bc	0.23±0.23 a
Punjab-91	0.92±0.09 a	0.71±0.03 a	4.36±0.69 ab	0.09±0.01 a
Bittle-98	0.63±0.02 a	1.30±0.31 a	4.19±0.51 a	0.10±0.02 a

Table 2: Coefficients of correlation

Parameters	No. of adults	No. of eggs	% Weight loss	No. of holes
No. of adults	1.000			
No. of eggs	0.920**	1.000		
% Weight loss	0.750**	0.668**	1.000	
No. of holes	0.875*	0.843**	0.847**	1.000**

Significant at 1% level of probability

Table 3: Chemical analysis of different chickpea varieties

	(%)						
Variety/ Genotype	Dry matter	Moisture	Crude protein	Crude fat	Crude Fiber	Total Mineral (ash)	Tanin
Parbat	89.00	11.00	16.62	2.00	17.50	2.50	1.202
Pb-2000	88.50	11.50	16.00	1.80	13.00	3.00	1.000
CM-2000	88.67	11.33	19.25	3.58	23.00	3.76	1.265
Paidar-91	88.83	11.17	17.50	4.27	19.00	3.21	1.000
Punjab-91	88.90	11.10	21.43	4.30	10.00	3.00	1.100
Bittle-98	89.10	10.90	17.50	1.70	23.00	3.50	0.730

Agtoxin, following Riaz [6] for two weeks so as to kill any pest already existing. The varieties were subjected to Antibiosis test after fumigation. Plastic jars of size (11x 9.5 cm) were used as experimental units. In all the jars 50 g of the chickpea genotypes were placed. The 10 pairs of 24 h old adult of CCL were released in each jar, following Halstead [7]. The treatments representing six varieties were replicated three times. Data on number of holes, number of adults, number of eggs and % age weight loss were recorded.

RESULS AND DISCUSSION

All the data were subjected to statistical analysis of variance using SPSS version 12.

Number of adults: The data collected on the number of adults of CCL in different gram varieties were subjected to statistical analysis and the results are given in Table 1. Table 1 shows that highly significant differences among the gram varieties regarding number of adults were observed. Maximum value was observed in Parbat. Paidar-91 showed medium number of adults. The other varieties showed minimum number of adults. Khattak [8] reported that none of the variety was completely resistant to CCL. Jha [5] reported that highest adult emergence (87.5%) was observed in BG 391.

Number of eggs: The results in Table 1 revealed that there were significant differences among all the varieties regarding number of eggs. Maximum number of eggs was laid by CCL on Parbat which significantly differed from others. Paidar-91 showed medium number of eggs, while all others showed minimum number of eggs. Jha [5] checked the response of chickpea against CCL and reported that attraction of CCL to different varieties varied.

Percent weight loss: Results given in Table 1 showed highly significant differences among varieties based on percent weight loss. The maximum weight loss was observed in Parbat which did not differ significantly from Paidar-91. CM -2000 did not differ significantly from Paidar-91 and Punjab-91. The lowest weight loss was observed in Bittle-98. Ahmad [10] reported significant differences among 47 varieties of chickpea for percent damage seeds, number of holes and seed coat texture after exposing to CCL.

Number of holes: Highly significant differences were observed in different varieties regarding number of holes. Maximum number of holes were observed in Parbat. Paidar-91 showed medium number of holes. In all other varieties the lowest number of holes were observed. Ahmad [9] studied different varieties of chickpea and reported that number of holes differed significantly.

When compared with susceptible/standard Paidar-91, Parbat proved to be highly susceptible against CCL. The CM-2000 proved to be susceptible. The varieties Punjab-91 and Pb-2000 proved to be partially resistant while Bittle-98 proved to be resistant against CCL. There was a positive significant correlation among number of adults and number of eggs, number of adults and percent weight loss, number of adults and number of holes, number of eggs and weight loss, number of eggs and number of holes, percent weight loss and number of holes which showed that increase in number of eggs and adults resulted in more weight loss and number of holes in grains. Chemical analysis of different varieties showed variations in dry matter, moisture, crude protein, fat, fiber, total mineral (ash) and Tanin (Table 3). Khattak [8] reported correlation coefficients between different variables showing highly significant values.

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Management of *Callosobruchus chinensis* Linnaeus in Stored Chickpea Through Interspecific and Intraspecific Predation by Ants

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Abstract: Predatory efficacy of three ant species i.e. *Monomorium minimum*, *Dorylus labiatus* and *Camponotus rufipes* was checked on all stages of *Callosobruchus chinensis* Linnaeus (CCL). *D. labiatus* was found to be the most efficient larvae and pupae predatory ant species with 84.65% and 98.26% rate of mortality of CCL larvae and pupae respectively. *M. minimum* was the most efficient egg eating species with 84.85% rate of CCL egg mortality. Considerable CCL adult predation was shown by *C. rufipes*. Forest lines of most of the species were better CCL predators. It was concluded that ants could be effective management tools against CCL.

Key words: Pulses % dhora % predators % pest % tropical crops

INTRODUCTION

Pulses are important sources of proteins, fats, carbohydrates, sugars and vitamin B. Gram (*Cicer arietinum*) is among one of the most important pulse crops famous as a good source of protein. In Pakistan, an area of 1088.8 thousand hectares was cultivated under gram during the year 2003-04 with a production of 667 thousand tons [1]. *Callosobruchus chinensis* Linnaeus (CCL) (Coleoptera, Bruchidae) commonly known as "Dhora" is one of the most destructive insect pests of this crop in stored conditions. According to Qayyum and Zafar [2] the maximum loss of 90% in gram due to CCL was reported. Heavy losses have been caused by CCL in moth beans [3]. Attempts have been made by different scientists for the biological control of stored Bruchid beetles through parasitoids.

The myrmicine genus *Monomorium* is one of the most important groups of ants in terms of its diversity, morphological and biological variability and its extensive range throughout the world. Several taxa, i.e. the pharaoh ant (*Monomorium pharaonis*, the Singapore ant (*Monomorium destructor*) and *Monomorium floricola* (Jerdon) are also notable tramp species and domestic pests [4]. On a world scale, Bolton [5] listed 296 species in the genus *Monomorium.* The ants are mostly considered pests of different commodities both in the fields and urban habitats [6]. In urban populations, ants cause frequent pest problems destroying aesthetic and economic value of many products of human consumption [7, 8]. Ants also act as natural predators of many pests. Ants have been observed to be one of the most common generalized predators in tropical crops. A few species may dominate high species richness in some crops [9]. Reznikova and Panteleeva [10] observed the active hunting of ants for springtails which are inhabitants of litter-soil stratum in various natural zones and landscapes. Ants are helpful in controlling a variety of insect pests in temperate and tropical crops, such as cocoa, pears, cotton, rice etc., [11-14]. A predatory ant, *Oecophylla smaragdina* (Fabricius) is the example of earliest biological control agent used in China in 300 AD for controlling Lepidopterous and Coleopterous pests of citrus. This practice is continued till date[7, 15].

The present studies were carried out to observe the predatory efficacy of different ant species on all developmental stages of CCL and to study the intra-specific variations in predatory efficacy among lines of most efficient predatory ant belonging to different colonies inhabiting domestic and forest habitats.

MATERIALS AND METHODS

A variety CM72 of chickpea was collected from Pulses Program, National Agriculture Research Center, Islamabad. It was fumigated using Agtoxin tablets for a

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period of two weeks. The culture of CCL was multiplied and maintained in mud rearing jars. Three ant species *Monomorium minimum*, *Dorylus labiatus* and *Camponotus rufipes* were collected from urban /semi-urban colonies and forests of different localities in Rawalpindi and Islamabad. This gave three so-called "lines" of each of the three species. Each group of ants was collected from the same colonies in order to maintain genetic uniformity among same species. The ant culture was maintained on sucrose sugar and aqueous honey solution (1:1 water and honey) in 2 kg capacity plastic insect rearing jars. The experiments were carried out under laboratory conditions at 30±2°C temperature and 65±5% relative humidity. All the experiments were laid out following Completely Randomized Design (CRD) and each treatment was replicated by three times.

Experiment I: Twelve plastic insect rearing jars of 200 g capacity were taken and 20 g of chickpea were added in each jar. Ten pairs of 24 h old CCL were transferred in each rearing jar following Halstead [16]. The mouths of jars were closed with muslin cloth and incubated. After one week CCL adults were removed and number of eggs laid were counted. Ten worker ants of semi-urban habitat of each species of ants i.e. *M. minimum*, *D. labiatus* and *C. rufipes* were added in rearing jars in three replications leaving three jars without ants as control. A cotton piece soaked in aqueous honey solution (1:1 water and honey) was placed in each of all twelve jars to provide supplemental food to ants ensuring their survival. The mouths of these jars were closed with the help of muslin clothes and incubated. Ants dying naturally were replaced by the fresh ones ensuring their specified number throughout the experimental period. Number of eggs of CCL consumed by ants were counted just before hatching and compared with control. Similarly number of larvae, pupae and adults developing from the surviving eggs and consumed by ants were also counted and compared with control.

Experiment II: This experiment consisted of three sub-experiments estimating ants directly feeding on CCL. Thirty plastic rearing jars were taken. Mouth closing and incubation of jars was same as in experiment I.

Sub-experiment-1 (Number of eggs consumed): In each of 30 plastic insect rearing jars 20 g of un-infested chickpea were added along with 24 h old ten CCL adult pairs and incubated. After one week, adults were removed and number of eggs laid were counted. Thirty ants belonging to three lines of all three ant species were added in insect rearing jars each in three replications. Three jars were left as control. After three days of incubation, number of CCL eggs consumed by the ants were counted.

Table 1: Percent mortality of *Callosobruchus chinensis* L. by various ant species

	Percentage Mortality of CCL			
Ant Species	Eggs	Larvae	Pupae	Adults
Camponotus rufipes	18.325 c	5.458 c	11.658 c	23.589 a
Monomorium minimum	84.854 a	62.985 b	53.458 b	4.325 b
Dorylus labiatus	51.214 b	84.652 a	98.265 a	2.645 b
Control	17.325 c	6.548 c	7.589 c	4.650 b

Sub-experiment-2 (Number of larvae/pupae consumed): In 30 insect rearing jars, 20 g of chickpea seeds with fifty larvae/ pupae were added along with thirty worker ants of different lines of three ant species each in three replications. Three jars were left as control. After three days of incubation, number of larvae/ pupae consumed by ants were counted.

Sub-experiment-3 (Number of adults consumed): In each of thirty insect rearing jars, 20 g of un-infested chickpea seeds were added along with 50 CCL adults. Thirty worker ants of three lines of all three species were added each in three insect rearing jars while three jars were left as control. After three days, number of adults consumed by ants were counted. Computer based *SPSS 10.0* and *Minitab V11* packages were used for data analysis.

RESULTS AND DISCUSSION

The results obtained from the experiments were expressed in the form of table and figures.

Inter-specific variation in predatory efficacy of ants: All of three ant species exhibited different levels of predation of different developmental stages of CCL as shown in Table 1.

***Egg predation*:** *M. minimum* consumed maximum number of eggs of CCL with the percentage mortality of 84.85% that was statistically different from all other ant species. It was followed by *D. labiatus* (51.21%). *C. rufipes* (18.32%) was similar to control (17.32%).

***Larvae predation*:** *D. labiatus* was proved to be the most efficient predatory ant species for CCL larvae with 84.65 percent mortality followed by *M. minimum* (68.98%). Again no significant CCL predation was shown by *C. rufipes* (5.45%) which was similar to control (6.54%).

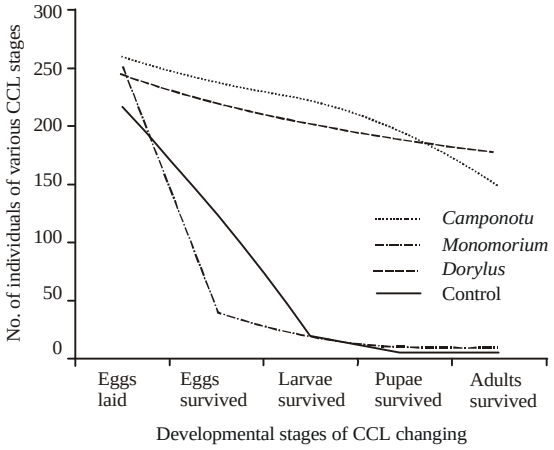

Fig. 1: Population dynamics of CCL influenced by ants

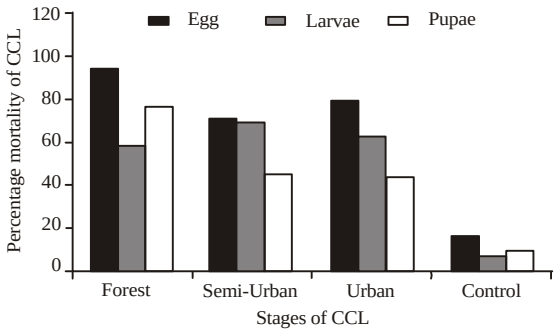

Fig. 2: Percent mortality of various developmental stages of CCL by various lines of *Monomorium minimum*

***Pupae predation*:** Maximum CCL pupae were predated by *D. labiatus* (98.26%) followed by *M. minimum* (53.54%). *C. rufipes* and control were statistically similar.

***Adult predation*:** Significant predation of adults of CCL was only shown by *C. rufipes* (23.58%). *M. minimum* (4.32%) and *D. labiatus* (2.64%) were statistically similar to control (4.65%).

***CCL population dynamics*:** The population dynamics of CCL influenced by the presence of various ant species is shown in Fig. 1. It was observed that *D. labiatus* and *M. minimum* decreased CCL population consuming different developmental stages at various levels of predation. *D. labiatus* killed maximum CCL at larvae and pupae level with 15 and 2% rate of survival respectively while *M. minimium* reduced pest pressure at the early egg stage where only 15% eggs survived. *C. rufipes* was similar to control only with the exception of notable adult predation where rate of adult survival was 76%. These results were similar to the conclusion of Adams *et al*., [9] that the presence of few ant species reduced the pest pressure being common predators of various crop pests.

Intra-specific variation in predatory efficacy of ants: *M. minimum* and *D. labiatus* did not show any significant adult predation of CCL so they were only analyzed for their egg, larvae and pupae predation in intra-specific studies.

***Predatory efficacy of Monomorium minimum*:** The results obtained were expressed in Fig. 2.

- **Egg predation:** Maximum CCL egg predation was shown by forest line of *M. minimum* (95.35%) that was statistically different from other two lines. Semi-urban (71.25%) and urban (79.25%) lines showed similar levels of CCL egg predation. Minimum egg mortality (15.35%) was observed in case of control.

- **Larvae predation:** Maximum CCL larvae predation was shown by semi-urban line (69.65%) but it was statistically similar to forest (58.32%) and urban (63.45%) lines while all three lines were different from control (6.57%).

- **Pupae predation:** Forest line of *M. minimum* exhibited maximum CCL pupae predation (77.154%) which was statistically different from semi-urban (45.65%) and urban (43.54%) lines. Control (9.71%) was different from all.

***Predatory efficacy of Dorylus labiatus*:** The results were shown in Fig. 3.

- **Egg predation:** Maximum number of eggs of CCL were predated by semi-urban line (47.21%) which was statistically different from other two lines. Urban (39.58%) was similar to forest line (34.25%). Control was different from all (18.26%).

- **Larvae predation:** Maximum larvae predation (92.47%) was shown by forest line of *D. labiatus* followed by urban line with 71.65% CCL larvae mortality. Semi-urban line (44.65%) was statistically different from both of first lines and control (8.29%).

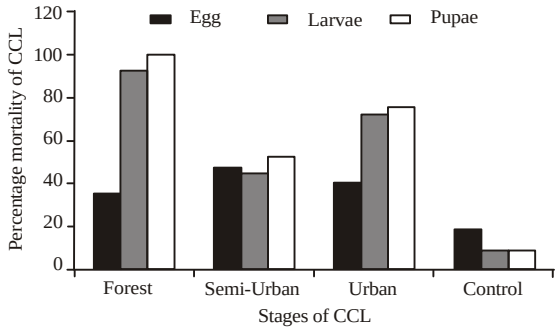

Fig. 3: Percent mortality of various developmental stages of CCL by various lines of *Dorylus labiatus*

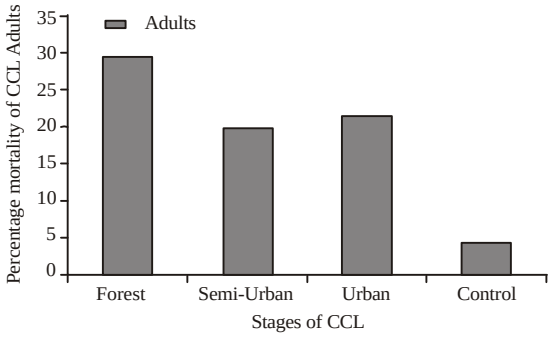

Fig. 4: Percent mortality of CCL adults by various lines of *Camponotus rufipes*

- **Pupae predation:** Hundred percent predation of pupae of CCL was shown by forest lines of *D. labiatus*. It was followed by urban line (75.25%) which was statistically different from semi-urban line (52.65%). Control was different from all (8.64%).

***Predatory efficacy of Camponotus rufipes*:** The results were shown in Fig. 4.

- **Adult predation:** Forest line of *C. rufipes* showed maximum predation of adults (29.21%) of CCL. Urban line (21.54%) and semi-urban line (19.85%). No significant egg, larvae or pupae predation of CCL was shown by *C. rufipes*.

It is obvious from the results that the ant species especially forest lines are potentially efficient predators of CCL as shown by various other field studies [10]. These ants can be used for the management of all stages of CCL especially eggs, larvae and pupae just like those ant species that have been used as natural predators of a variety of insect pests of temperate and tropical crops like cotton, pears, cocoa and rice [11-14]. If the storage buildings for chickpea are constructed in forest habitat these ants rather than becoming a pest, would benefit the farmers by eliminating CCL which is an economic insect pest of chickpea.

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Levels of Polyaromatic Hydrocarbons in Egyptian Vegetables and Their Behavior During Soaking in Oxidizing Agent Solutions

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Abstract: The residues of persistence potentially toxic organic compounds polyaromatic hydrocarbons (PAHs) were measured in 13 species of edible vegetables collected from Great Cairo governorate (Egypt). And the efficient role of washing by oxidizing agents hydrogen peroxide (H_2O_2), potassium permanganates ($KMnO_4$) and midribs of cabbage leaves solutions on the elimination of PAHs from contaminated vegetables was investigated. Vegetable samples were all soxhlet-extracted in triplicate, cleaned up by open chromatography and analysed using gas chromatography with mass selective detection. It was observed that all tested vegetables have been contaminated with PAHs. The total content of 16 PAHs ranged from 1.22 to 12.63 ppb with the highest PAHs found in leafy vegetables. PAHs (3-4 rings) were dominant in all vegetable samples. The results also indicates the efficient role of washing by oxidizing agent in elimination of PAHs from naturally contaminated carrots. Midribs of cabbage leaves solution is the most efficient in removing PAHs followed by H_2O_2 and $KMnO_4$. the removal percentages of the total PAHs ranged from 65.5 to 97.8% and from 79.4 to 99.9% at 4 and 8% concentrations of oxidizing reagents, respectively.

Keywords: PAHs %oxidizing agents % Vegetables

INTRODUCTION

Polyaromatic hydrocarbons (PAHs) refer to a large group of organic chemicals containing two or more fused aromatic rings made up of carbon and hydrogen atoms as a by-products from the incomplete combustion or pyrolsis of organic materials. Gaseous and particle-bound PAHs can be transported over long distances before deposition and may accumulate in vegetation. This could indirectly cause human exposure to PAHs through food consumption and, thus might pose a human health threat [1]. PAHs are ubiquitous in the environment being present in air, soil, water and food [2, 3]. The effect and fate of PAHs in nature are of great environmental and human health concerns due to their widespread occurrence, persistence in terrestrial ecosystems and carcinogenic properties as well as to have cardiovascular, bone marrow or liver toxicity [4, 5]. Since carcinogenicity is the critical endpoint of toxicity of PAHs and some PAHs e.g benzo[a]pyrene, benzo[a]anthrathene and dibenzo[a,h]anthracene are genotoxic, it is not possible to define a level of intake which is without possible [6, 7].

It has been-established that there are two major sources of PAHs formation in foods the first source is mainly due to the method of food preparation. The other major source of contamination of foodstuffs is by contact with either petroleum products or coal tar products. Because of the geochemical process and atmospheric deposition of air pollution particulate on the crops, it would be possible to generate these naturally occurring PAHs in foods [8]. As PAHs are ubiquitous in the environment, it is not surprising that they present in almost all foods [7]. For example, it has been reported that PAHs are present in creals, grains, flour, bread, vegetables, fruits, fish, meat, processed or pickled foods and contaminated cows milk or human breast milk [9-11]. Cereals and cereals products, milk, vegetables and fruits are the highest contributors to total PAH intake. Since these products are the most important dietary component. In Egypt until now studies dealing with the detection of PAHs residues in vegetables are still lacking. Therefore this study was performed to determine the levels of PAHs grown in Great Cairo Governorate and provide a method for efficient, economical and rapid for decontamination of fresh vegetables naturally contaminated with PAHs using some of oxidizing agent solutions.

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MATERIALS AND METHODS

Samples: Total of 130 vegetables samples belonging to 13 different species (lettuce, leak, green onion, Spinach, spearment, pepper, squash, eggplant, cucumber, tomatoes, sweet potatoes, potatoes and carrot) were collected randomly from different regions in Great Cairo Governotate (Cairo, Giza and Kalubia). All samples were collected during the period of January, 2003 up to July 2004 to determine the concentrations of PAHs. Twenty kilogram of carrots were collected from the local market in Great Cairo. A representative sample of about 2 kg was examined for PAHs and washing treatments were carried out on carrot samples previously naturally contaminated with PAHs. All of the samples were stored in amber bottles in a freezer until analysis.

Standards and chemicals: The mixed standard solution of 16 PAHs (naphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthrathene, fluornthene, pyrene, benzo[a]anthracene, chrysene, benzo[b] fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3,c,d]pyrene were purchased from Qmx Laboratories Limited, UK.

All solvents and chemicals used were obtained from E. Merck Company (Germany). Midribs of cabbage leaves solutions were prepared by cutting it into small species and soaking in water at 4 and 8% concentrations.

Extraction, clean-up and analysis: PAHs were quantified from Soxhlet extract of each vegetable sample. In brief, 30 g each sample was mixed with 90 g of anhydrous sodium sulphate and placed in pre-extracted Whatman extraction thimbles (43 mm x 123 mm). The thimbles were placed into a 500 mL Quiekfit Soxhlet unit and extracted for 24 h with 300 mL of hexane:acetone (1:1). Extracts were then reduced to near dryness on a rotary film evaporator (Buchi R-124), taken up in 10 mL hexane and transferred into pre-washed and baked glass vials. The samples were then reduced further on a Techne Dri-Blok under a gentle stream of N_2 to 2 mL. The extracts were cleaned by passing through Bakerbond PCB-A SPE cartridges pre-conditioned with 10 mL hexane. They were again reduced under N_2 to near dryness, transferred to clean glass vials and made up to 1 mL for analysis. One µL of each clean sample extract was injected into an Hewlett Packarel 6890 gas chromatograph fitted with a 30 m HP5-MS fused silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness) and connected to an Hewlett Packard 5973 mass selective detector. The carrier gas was helium, maintained at a flow rate of 1.0ml/min by electronic pneumatic control. The injection port temperature was 230°C with electron energy of 70 eV. The quadrupole temperature was 280°C. The instrument was tuned on PFTBA. The oven programme for PAHs was as follow: 90°C for 2 min, 10°C/min to 240°C, 3°C/min to 310°C for 5 min. The mass spectrometer was operated in selective ion monitoring (SIM) mode using separate ions to identify and confirm compounds.

Treatment of contaminated carrots: The contaminated intact carrot samples were separately soaked for 10 min in potassium permanganate, hydrogen peroxide and midribs of cabbage leaves solutions at concentration of 4 and 8%. The samples were packed in amber glass bottles and stored in a freezer until analysis.

RESULTS AND DISCUSSION

The results in Table 1 shows that all tested vegetables have been contaminated with PAHs with different levels ranging from 1.22 to 12.63 with a mean value 6.23 ppb. It was notice from the results that the 3-4 rings PAHs were predominates in all samples. However phenanthrene and flouranthene are the most abundant individual PAH compounds in vegetables under investigation. These compounds are more water-soluble than the higher molecular weight PAHs and so may be more susceptible to uptake from soils as well as deposition from polluted air. The higher PAHs content in green leafy vegetables such as lettuce 12.63 ppb, leak 10.71 ppb, green onion 10.09 ppb and spinach 8.87 ppb could be explained by their greater contact surface to the ambient air during growth. Lin *et al*., [1] reported that tea leaves possess high surface area, so they may accumulate PAHs, especially from air. Lodovici *et al*., [12] found that all vegetables except tomatoes investigated in Italian study exposed to polluted air, contained high levels of carcinogenic PAH. In addition, in a selection of reported benzo[a]pyrene concentrations in food by Greenberg *et al*., [13] the level in kale was in a range of 12.6-48.1 ng/g. Kazerouni *et al*., [8] found that the highest level Bap was found in collards and kale with levels of 0.48 and 0.47 ng/g, respectively. This indicate that PAH levels in uncooked food largely depend on the origin of the food and can be subject to regional variations.

Investigation into the source of PAHs have been used the molecular ratios of some specific hydrocarbons [1]. For instance a ratio of fluornthene to pyrene concentrations (Fluor/pyr) greater than 1.0 were

Table 1: Levels of PAHs in vegetables collected from Great Cairo governorate

	Mean concentration of PAHs ppb[a]												
	Vegetables												
Compounds	Lettuce	Leak	Green onion	Spainch	Spearment	Squash	Cucumber	Eggplant	Pepper	Tomato	Carrot	Potatoes	Sweetpotatoes
Naphthalene	0.150±0.10	nd[b]	0.01±0.010	0.83±0.56	0.48±0.21	0.03±0.02	0.05±0.03	nd	0.06±0.48	nd	0.21±0.10	0.07±0.05	nd
Acenaphthalene	0.070±0.04	0.06±0.03	0.013±0.007	nd	nd	nd	nd	nd	0.04±0.03	nd	0.053±0.041	0.027±0.01	nd
Acenaphthene	0.810±0.36	1.30±0.81		0.6±0.5	1.01±0.8	nd	nd	nd	0.65±0.60	0.01±0.008	0.9±0.63	0.58±0.35	0.24±0.14
Fluorene	0.630±0.43	1.50±0.60	0.55±0.38	0.7±0.3	0.57±0.4	0.06±0.04	nd		0.43±0.50	nd	0.82±0.56	0.77±0.52	0.15±0.05
Phenanthrene	1.380±0.80	3.50±1.10	1.00±0.8	2.53±1.8	0.79±0.55	1.62±1.0	0.2±0.1	2.17±0.9	1.35±0.7	0.68±0.52	1.4±1.03	1.23±0.74	0.73±0.51
Anthrathene	1.050±0.90	0.96±0.58	0.83±0.53	0.74±0.61	0.45±0.27	0.13±0.10	0.03±0.02	0.01±0.01	0.056±0.03	0.03±0.02	0.35±0.42	nd	nd
Fluornthene	4.230±1.50	2.13±0.95	5.26±2.85	1.5±0.8	3.00±0.93	1.01±1.53	0.72±0.56	0.43±0.31	1.46±0.60	0.25±0.12	1.1±0.90	1.85±0.75	1.00±0.68
Pyrene	1.600±0.82	0.73±0.59	nd	1.02±1.00	0.20±0.08	1.2±0.72	0.05±0.04	0.09±0.05	1.07±0.9	0.13±0.07	0.78±0.81	1.00±0.65	0.56±0.37
Benzo[a]anthracene	0.520±0.51	0.41±0.28	0.19±0.08		0.03±0.019	0.17±0.09	0.01	0.1±0.09	0.4±0.31	0.06±0.04	0.16±0.2	0.34±0.28	0.023±0.01
Chrysene	0.110±0.05	nd	0.21±0.11	0.25±0.3	0.124±0.11	nd	0.08±0.05	0.12±0.05	0.13±0.10	nd	0.08±0.07	0.07±0.05	nd
Benzo[b]fluoranthene	0.240±0.17	0.01±0.01	0.35±0.30		0.15±0.08	nd	nd	nd	nd	nd	nd	nd	nd
Benzo[k]fluoranthene	0.198±0.08	nd	0.33±0.19	0.13±0.07	0.328±0.23	0.09±0.05	nd	0.01±0.008	nd	nd	nd	nd	nd
Benzo[a]pyrene	0.610±0.39	0.02±0.01	0.29±0.15	0.54±0.50	0.132±0.07	0.04±0.03	0.07±0.05	0.02±0.02	0.16±0.07	0.01±0.005	0.15±0.12	0.03±0.23	nd
Indeno[1,2,3-c,d]pyrene	0.280±0.12	nd	0.15±0.07	nd	0.07±0.06	nd	nd	nd	nd	nd	0.11±0.20	nd	nd
Dibenzo[a,h]anthracene	0.550±0.26	0.06±0.04	0.14±0.08	nd	0.035±0.013	0.05±0.02	nd	nd	nd	nd	0.02±0.013	nd	nd
Benzo[g,h,I]perylene	0.200±0.17	nd	0.267±0.20	nd	0.028±0.02	0.2±0.08	nd	nd	0.05±0.03	nd	0.34±0.33	nd	nd
?E PAHs	12.628±1.025	10.705±1.006	10.095±1.258	8.87±0.694	7.402±0.732	4.625±0.972	1.245±0.248	3.035±0.539	5.876±0.501	1.215±0.174	6.483±0.446	5.997±0.515	2.753±0.311

[a]Limit of detection is 0.005 ppb, [b]nd, not detectable

Table 2: Behavior of PAHs residues during soaking in oxidizing solutions for 10 min

	Treatments											
		$KmnO_4$				H_2O_2				MCL		
	Control	4%		8%		4%		8%		4%		8%	
Compounds	[a]Mean	Mean	Reduction (%)	Mean	Reduction (%)	Mean	Reduction (%)	Mean	Reduction (%)	Mean	Reduction (%)	Mean	Reduction (%)
Naphthalene	0.23±0.05	0.09±0.03	60.9	0.07±0.023	69.6	0.06±0.017	73.9	0.05±0.01	78.3	nd	100.0	nd	100.0
Acenaphthalene	0.06±0.015	0.02±0.005	66.7	0.014±0.005	76.7	0.009±0.001	85.0	[b]nd	100.0	nd	100.0	nd	100.0
Acenaphthene	0.82±0.18	0.26±0.07	68.3	0.15±0.037	81.7	0.2±0.06	75.6	0.10±0.03	87.8	nd	100.0	nd	100.0
Fluorene	0.53±0.12	0.22±0.08	58.3	0.14±0.03	73.6	0.19±0.05	64.2	0.08±0.03	84.9	0.05±0.01	89.2	nd	100.0
Phenanthrene	2.17±0.45	0.81±0.23	62.7	0.56±0.19	74.2	0.85±0.25	60.8	0.51±0.13	76.5	0.023±0.005	96.9	nd	100.0
Anthrathene	0.75±0.15	0.28±0.06	62.7	0.13±0.03	82.7	0.17±0.036	77.3	0.07±0.018	90.7	nd	100.0	nd	100.0
Fluornthene	1.5±0.27	0.5±0.16	66.7	0.28±0.09	81.3	0.36±0.09	76.0	0.21±0.07	86.0	0.07±0.02	93.5	nd	100.0
Pyrene	1.08±0.33	0.4±0.17	63.0	0.19±0.053	82.4	0.29±0.07	73.1	0.13±0.04	88.0	0.01±0.001	95.8	nd	100.0
Benzo[a]anthracene	0.24±0.08	0.05±0.02	79.2	0.027±0.006	79.2	0.015±0.003	93.8	nd	100.0	nd	100.0	nd	100.0
Chrysene	0.13±0.04	0.04±0.013	69.2	0.023±0.007	82.3	0.011±0.004	91.5	nd	100.0	nd	100.0	nd	100.0
Benzo[b]fluoranthene	0.06±0.01	0.015±0.003	75.0	0.009±0.002	85.0	0.013±0.002	78.3	nd	100.0	nd	100.0	nd	100.0
Benzo[k]fluoranthene	0.124±0.03	0.034±0.012	72.6	0.02±0.007	83.9	0.025±0.006	79.8	0.014±0.003	88.7	nd	100.0	nd	100.0
Benzo[a]pyrene	0.548±0.14	0.161±0.035	70.6	0.089±0.03	83.8	0.12±0.03	78.1	0.05±0.013	91.0	nd	100.0	nd	100.0
Indeno[1,2,3-c,d]pyrene	0.11±0.03	0.028±0.006	74.5	0.017±0.004	74.5	0.018±0.005	83.6	0.007±0.002	93.6	nd	100.0	nd	100.0
Dibenzo[a,h]anthracene	0.06±0.03	0.013±0.013	78.3	0.008±0.003	86.7	0.015±0.005	75.0	0.006±0.001	91.7	nd	100.0	nd	100.0
Benzo[g,h,I]perylene	0.376±0.09	0.112±0.08	70.2	0.085±0.021	77.4	0.09±0.018	76.1	0.062±0.015	83.5	0.04±0.01	89.4	nd	97.3
E PAHs	8.788	3.033		1.812		2.436		0.283		0.193		0.010	
Reduction%			65.5		79.4		72.3		96.8		97.8		99.9

[a]Mean = ppb±SD, Limit of dection is 0.005ppb, Values given are mean of five replicates, [b]nd, not detectable

characteristic of pyrolytic origin, wheras ratio less than 1.0 was characteristic of petroleum hydrocarbons [14]. And a ratio of phenanthrene to anthracene (Phen/An) less than 10 suggested combusion sources, while Phen/An greater than 10 implied petrogenic sources [15, 16]. From the calculation of Flur/pyr and Phen/An in vegetables tested in this study it was indicate that PAHs resulting from incomplete combustions products via pyrolytic process from industrial plants and from farm waste and home waste fires in addition to atmospheric fall out of automobiles exhausts in cities and town along the road of Great Cairo governorate.

The effect of washing by oxidizing agent solutions on the the removal of PAHs were summarized in Table 2. The results indicates the efficient role of washing by oxidizing agents solutions, Midribs of cabbage leaves (MCL), hydrogen peroxide (H_2O_2) and potassium permanganate KMnO4 in reduction of PAHs from naturally contaminated carrots. On the other hand, washing with tape water not provided significant effective loss with the contamination by PAHs. It was noticed that reduction of these contaminants depends on the type and levels of PAHs individuals as well as the type and concentration of oxidizing agents. It appears from the results that MCL is the most efficient followed by H_2O_2 and $KMnO_4$ in removing PAHs contamination. MCL solution at 4% concentration completely eliminated acenaphthylene, acenaphthene, fluorene, fluoranthene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene and dibenzo[a,h]anthrathene. And giving 89.2, 96.9, 93.5 and 95.8% reduction in phenanthrene, anthrathene, pyrene, benzo[a]anthrathene and benzo[ghi]perylene. At 8% concentration MCL eliminated PAHs residues completely except for benzo[ghi]perylene (97.3% loss). This may be explained on the basis that MCL has the ability to excert peroxidase enzyme [17]. It was found that peroxidase has the ability to breaks PCBs [18].

The results in Table 2 also show a significant decrease in the levels of PAHs under study by washing with H_2O_2 and $KMnO_4$ solutions at 4 and 8% concentrations. The levels of total PAH reduction were 65.5, 79.4, 72.3, 96.8%, respectively. The redox potentials of H_2O_2 and $KMnO_4$ are high (-1.736 and -1.507 v, respectively) and therefore are highly reactive towards PAHs. The pathway by which H_2O_2 breaks down aromatic ring compounds involves highly free radicals (e.g. HO_2 and HO) [19].

It could be concluded from the above results that Egyptian vegetables contaminated by different levels of PAH individuals; this contamination may be due to pollution of the environment and deposition in plants. So more efforts should be applied by the Ministry of Environment to find effective ways to control the environmental pollutions. Washing with oxidizing agent solutions show a significant effect on decomposition and removal of PAHs from contaminated vegetables and therefore they are necessary for kitchen use to decrease the intake of PAHs residues. And so, decrease from the possible health hazard arising from the toxic PAHs residues in food.

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Reproduction of *Poecilocerus bmtonius* fed on *Calotropis procera* Compared with *Zygophylum simplex* and *Pulicaria crispa*

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Abstract: Feeding of adult grasshopper, *Poecilocerus bmtonius* on three host foliage; *Calotropis procera*, *Zygophylum simplex* and *Pulicaria crispa* was investigated by measuring immature and mature female longevities, weight gain, fertility and fecundity. The development of adult females were shorter on *C. procera* than those fed on other host foliages. Fecundity and fertility of females fed on *C. procera* were significantly higher compared with those reared on *Z. simplex* and *P. crispa*. Ovariole yield of females reared on *Z. simplex* or *P. crispa* was 0.0% as compared with females reared on *C. procera*. The rate of resorption bodies per ovary for females reared on *Z. simplex* or *P. crispa* was 100%. Weight gain in male or female adults reared on *C. procera* was higher than those reared on the other host foliages.

Key words: Feeding % fertility % grasshopper % longevities

INTRODUCTION

The grasshopper, *Poecilocerus pictus* eject a defensive fluid as an escape- measure against the attack of predators [1]. For this reason, the grasshopper, *Poecilocerus bmtonius* is more attracted to *Calotropis procera* plants for accepting its chemical defensive fluid (cardenolides), using it as a chemical defense against natural enemies. Plants use cardenolides against natural enemies including many herbivorous animals, parasites and pathogens [2].

The highest percentage of *Schistocerca gregaria* egg hatch was recorded from parents feeding on *Chrozophora penniseturn*, while no egg hatch occurred when parents were fed on Sorghum plants. Maturation of *S. gregaria* are adversely affected by feeding on *Tribulus*, *Sorgum* and *Dipterygium* [3]. But Rao [4] found that *Tribulus terrestris* allowed rapid maturation.

The importance of the various components of host plant in an insect's diet can be determined by assessing nymph growth rate and food utilization [5, 6]. Rearing the grasshopper, *Euprepocnemis plorans* on lupine and horse bean caused significant reduction in fertility and ovariole yield of its first and second pods compared with those reared on clover [7].

The objective of the present study is to determine the effects of feeding foliages of the two host plants, *Zygophylhvn simplex* and *Pulicaria crispa* on the reproduction of this grasshopper and evaluate its influence when the main host, *C. procera* is absent.

MATERIAL AND METHODS

Egg masses were collected from routinely reared grasshoppers and incubated at 33°C in a closed jar until eclosion. Newly hatched nymphs were maintained on fresh mixed foliages from *C. procera*, *Z. simplex* and *P. crispa* at 30°C and 50-70% RH and a 12:12 (L:D) photoperiod. Leaves of *C. procera*, *Z. simplex* and *P. crispa* were collected from the field and dried, then offered to test insects.

Newly emerged pairs originating from a single egg mass were individually reared in a 1-L glass jar covered with a cap fitted with a metal grid. A Petri dish containing the tested plants and a wet piece of cotton as a source of water was placed in the jar. Eight pairs were reared on each of the three foliages. New dried leaves and wet cotton were provided daily. The glass jars were filled with moist sand after two weeks and egg pods laid by the mated females were collected. Egg pods were counted and the sand was replaced. Egg pods were incubated at 30°C until hatching. The number of egg pods per female, eggs per pod, hatched nymphs per pod, the incubation period, mean number of ovarioles per ovary, resorption bodies per ovary and fecundity were recorded for each host foliage using the following formulas:

Fecundity = Total number of eggs per female

$$\text{Ovariole yield (\%)} = \frac{\text{No. of eggs per pod}}{\text{No. of ovarioles eggs per female}} \times 100$$

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Table 1: Weight gain (fresh weight in grams) and ovary development of *P. bmtonius* fed on *C. procera, Z. simplex* and *P. crispa* (means + SD)

	Weight of immature adults		Weight of mature adults		Weight gain		No. of ovarioles	Resorption bodies per
Host	Females	Males	Females	Males	Females	Males	per ovary	ovary
C. procera	1.83±0.25	1.26±0.34	3.35±0.27	2.10±0.36	1.52±0.02	0.84±0.03	121.0±8.7	35.0±2.9
C. Simplex	2.10±0.62	1.29±0.36	2.31±0.56	1.40±0.33	0.21±0.06	0.11±0.02	71.3±26.9	100.0±0.0
P. crispa	1.73±0.25	1.30±0.13	1.89±0.25	1.40±0.10	0.16±0.01	0.10±0.03	97.8±18.1	100.0±0.0

Table 2: Percentage of fecundity, fertility and ovariole yield of of *P. bmtonius* fed on *C. procera, Z. simplex* and *P. crispa* (means + SD)

Host	No. of pods/ female	No. of eggs/ pod	No. of hatched eggs/ pod	Fecundity (%)	Fertility (%)	Ovariole yield (%)	Duration of incubation in days	Period between two layeings
C. procera	3.5±0.54	113.9±8.64	95.0±4.50	94.9±2.52	83.55±2.9	94.1+2.26	27.5±1.60	13.6±0.74
Z. Simplex	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
P. Crispa	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00

$$\text{Fertility (\%)} = \frac{\text{No. of hatched eggs per pod}}{\text{No. of deposited eggs per pod}} \times 100$$

Weight gain and resorption bodies: Table 1 shows that the weight gain of male and female adults reared on *C. procera* was significantly higher than those reared on *Z. simplex or P. crispa*. Also, thc parentage of resorption bodies per ovary in females fed on *Z. simplex* or *P. crispa* was 100% but it was 35% in the ovary of females fed on *C. procera*. The mean number of ovarioles per ovary in females reared on *C. procera* was higher than that of females fed on *Z. simplex* or *P. crispa*.

Fecundity, fertility and ovariole yield: Number of pods per female, number of eggs per pod and number of hatched eggs per pod were significantly higher in females fed on *C. procera*, but females reared on *Z. simplex* or *P. crispa* laid no eggs (Table 2). The percentage of fecundity, fertility and ovariole yield was normal in females fed on *C. procera.* Incubation period of eggs was 27 days and the period between two laying was 13 days.

Delaying of the sexual maturation of adult females reared on *Z. simplex* or *P. crispa* is attributable to ovary weakness. Ellis and Carlisl [8] showed the storage of certain nutrients, notably gibberellins and monoterpenoids in senescent vegetation may lead to delayed maturation. Negative effects resulting from dietary components in *Lupinus termis* or *Vicia faba* caused prolongation of developmental period [7]. Similar observations were recorded after feeding *Schistocerca gregaria* on *Schouwia purpurea* [9]. *S. gregaria* on *Pennisetum*, *Dipterygium*, *Tribulus* and *Chrozophora* plants supported rapid growth and development [3].

Decrease of weight gain in the male and female adults of grasshopper on *Z. simplex* or *P. crispa* may be due to an effect on protein synthesis. This probability was confirmed when dissected females showed resorption bodies in ovarioles, the percentage were100%. Low growth rate of *Ligurotetix coquilletti* female grasshopper on avoided shrubs was attributable to the low conversion rate of the digested food [10]. The grasshopper, *Melanoplus sanguinipes* growth was significantly lower on kochia. Weight was reduced and duration of development increased on oats and kochia plant [11, 12]. Increase of egg reabsorption % may depend on haemolymph proteins and fat bodies in female's or *S. gregaria* on *S. purpurea* [9]. Resorption bodies were significantly higher in the ovarioles of *E. plorans* females fed on *L. termis* or *V. faba* [7].

No reproduction which was recorded in the females reared on *Z. simplex* or *P. crispa*, may be due to failure to complete maturation of ovarioles or/and failure of production of yolk proteins in the haemolymph resulting from injurious components in these host plants. The percentage of egg resorption was 100% in females fed on *Z. simplex* or *P. crispa*. Fecundity and fertility of the grasshopper, *E. plorans* fed on clover were significantly higher than those of females fed on either lupine or horse bean [7]. Hatchability was 0.0% on *sorghum sp*. [13]. Percentage of reproduction of the grasshopper *Melanoplus sanguininipes* on kochia and oats was lower than that on wheat [11, 14]. The highest percentage of egg hatching in *S. gregaria* was recorded with parents feeding on *Chrozophora* and *Pennisetum* but it was 0.0% on Sorghum plants [3].

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Diallel Analysis of Cassava Genotypes to Anthracnose Disease

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Abstract: Cassava anthracnose disease (CAD) caused by *Colletotrichum gloeosporioides* f. sp *manihotis* has been recognized as one of the major economic disease of cassava in all the cassava growing regions of Africa. Little information is available on the resistance of cassava to *C. gloeosporioides* f. sp *manihotis*. This study was conducted to determine the relative importance of general (GCA) and specific (SCA) combining ability, maternal and non-maternal reciprocal effects on resistance *to C. gloeosporioides* f. sp *manihoti*s in selected cassava genotypes. A complete diallel mating scheme including reciprocals of nine resistant and susceptible genotypes of cassava were evaluated in the field over a period of two planting seasons. The combining ability analysis revealed that both the additive and nonadditive gene effects were present. Crosses between the resistant lines and susceptible genotypes showed intermediate disease reaction to CAD suggesting a polygenic system of resistance to the disease. The significant maternal and specific reciprocal differences among the parents and crosses indicated that maternal and/or cytoplasmic inheritance is involved in the reaction of cassava genotypes to *Colletotrichum gloeosporioides* f. sp *manihotis*. The significant genotype X environment interaction suggested lack of stability in the development of lesions/cankers on cassava stems. Therefore, recurrent selection would be appropriate for accumulating genes for resistance to CAD in cassava and progeny performance may not be based on their parents performance per se.

Key words: *Collectotichum gloeosporiodes* f. sp *manihotis*, combining ability, diallel, *Manihot esculenta*, resisitance

INTRODUCTION

The tropical root crop cassava (*Manihot esculenta* Crantz) is the third most important source of calories for human food in the tropics after rice and maize. Over 600 million people depend on cassava in Africa, Asia and Latin America. Cassava is grown by poor farmers, many of them are women. For these people, the crop is vital for both food security and income generation [1]. In spite of the importance of this crop as a famine and food security plant, it is constantly threatened by production constraints such as drought, low yielding local cultivars, lack of good quality planting materials, land tenure, pests and diseases. Lozano *et al*. [2].

Of all the diseases found on cassava, Cassava Anthracnose Disease (CAD) caused by *Colletotrichum gloeosporioides* Penz f. sp *manihotis* Chev is the most important fungal disease of cassava in the field [3]. The most outstanding effect of the disease is its ability to cause severe stem damage causing canker on stem, wilting of leaves and diebacks. Badly infected stems become brittle and break easily under strong winds. The overall effect of these is the reduction in yield and in the amount of healthy plantable stems available to the farmers. The frequency with which the disease is encountered in cassava in African has been a matter of concern to many workers. Muyolo [4] and Makambila [5] reported that between 80-90% of local cultivars were rated as severely infected in Zaire and Congo, respectively. Fokunang [6] also observed that the causal organism of CAD was found on cassava stems from all the humid and the sub-humid agro-ecological zones of Nigeria, just as Wydra and Msikita [7] reported a high incidence of CAD across the countries of the rainforest and transition forest zones. In spite of all these reports of widespread distribution of CAD in Africa and the progress made in resistance breeding to Africa Cassava Mosaic Virus (ACMV), Cassava Bacterial Blight [8, 9], CAD is rarely taken into consideration in the breeding programme. The spotlight has, therefore, shifted to host plant resistance since it is acknowledged that, resistant cassava varieties could potentially form the basis of sustainable

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management strategies for cassava diseases [8 - 10]. The selection of resistant varieties and continuous breeding programme for disease resistance appears to be the efficient means of controlling CAD. Although little work has been done on resistance to CAD and determination of mode on inheritance. Studies in these areas will assist the breeder in formulating an efficient strategy for incorporating the resistant genes into high yielding improved and stable varieties. The overall objective of the present work is to contribute to the development of stable anthracnose resistance in cassava. The specific objective of this study was to evaluate the relative importance of general and specific combining ability for resistance to CAD.

MATERIALS AND METHODS

Genetic experiments: The present study was carried out on the experimental fields of the International Institute of Tropical Agriculture (IITA) at Ibadan in Nigeria. The genetic materials were evaluated using the complete diallel mating Scheme during 2003 and 2004 growing seasons. The genotypes were selected based on plant vigour (PV), flowering ability (FA) and sprouting ability (SA).

All possible crosses involving 9 parents with various degrees of resistance to CAD; (resistant, moderately resistant and susceptible varieties) were made on IITA research field in Ubiaja, Edo State, Nigeria in 2001 by hand pollination and the seeds were made available by cassava breeding unit at IITA for the present study. The seeds were planted in pots in nursery prior to transplanting in the field and watered twice daily for three weeks and then once a day until the seedlings were established. Established seedlings were transplanted in the field at the same time to produce woody cuttings for the study. Mature stakes (25 cm long) of the parents were planted at the beginning of the rainy season (June 2003). A Randomized Complete Block Design with three replicates was used. Each plot consisted of a minimum of 40 plants spaced 0.5 m apart in rows (ridges 30 cm high and 10 m long) and were spaced 1m apart, giving a plant population of 20,000 plants per hectare. No fertilizer or herbicide was applied during the course of the experiment and hand weeding were done when necessary. The parents and their F_1's hybrids were evaluated under rain fed conditions in an area known for CAD epidemics in the year 2003 and 2004 at IITA's research farm in Ibadan, Nigeria, for their reactions to CAD 12 MAP. The improved cassava genotypes TMS I30572 (highly susceptible), TMS 91/02324 (moderately resistant), a moderately resistant landrace TME 117 (Isunkankiyan) and a susceptible landrace TME 1 (Antiota) were included as checks.

Individual plants were examined for symptom severity using the parameters and method as adopted by Ikotun and Hahn [11].

Genotypes were partitioned into variation due to lines (parents and crosses) and checks using the GLM procedure in Statatistical Analysis System (SAS). Analysis of variance for the crosses was based on Griffing's method 2, model 1 for fixed genotypes [12], and the linear model [13]. The analysis was performed on individual environments using the diallel-SAS programme written by Kang [14] and a combined analysis over environments using the diallel-SAS programme written by Zhang and Kang [15].

The genaral linear model for an individual environment was:

$$yijk = \mu + g + gj + sij + rij + bk + m_{ijk}$$

where; yijk was the response of the kth observation in the ith environment of the plant; μ was general mean; gi the general combining ability (GCA) of the ith parent; gj the general combining ability (SCA) of the jth parent; sij the specific combining ability associated with the ith and jth cross; rij the reciprocal effects associated with ijth cross. bk the effect of the kth replicate and mijk is the error associated with each observation.

The general linear model for the combined analysis was:

$$Yijkl = \mu + gi + gj + sij + lk + bl(k + glik + gljk + slijk + mijl)$$

In this model, Yijkl was the observed response to CAD across the two seasons; μ, gi, gj and sij and its partitions mi and nj were for the individual season analysis. The effect lk was the effect of the kth season; bl(k) the effect of the lth replicate within the kth season. The effect glik was the general combining ability of the ith parent in the season; gljk the general combining ability of the jth parent in the kth environment and slijk the specific combining ability associated with the ijth cross in the season.

Genetic components of the variation associated with GCA and SCA effects were estimated from their respective expected means squares. The ratio of these components was computed to estimate the relative importance of GCA in predicting progeny performance. The GCA and SCA effects and their standard errors were estimated according

Table 1: Diallel analysis of variance for anthracnose disease of cassava

Sources of variation			df	Combined	2003	2004
Reps			2	15.45'	14.00'	13.23'
Environment (E)			1	36.36**		
Reps (E)			4	23.85'		
Genotypes (G)			80	143.36**	400.50**	224.7**
	Parent (P)		8	87.67**	204.00**	154.2**
	Cross (C)		71	137.24**	214.26**	225.04**
		SCA	27	405.49**	415.06**	344.37**
		GCA	8	156.40**	139.50**	165.1*
		Maternal	8	160.70**	192.04**	178.42**
		Reciprocal	36	32.55**	66.13**	80.15**
	P X C		1	39.42*	85.00**	60.40**
	C X E		71	49.55**		
		GCA X E	8	45.60**		
		SCA X E	27	126.57**		
		REC X E	36	24.82'		
		M X E	8	64.73**		
Error pooled			284	25.49'	17.05'	33..93'

*, ** Significantly different from zero at 0.05 and 0.01 probability levels, respectively

Table 2: Estimates of general combining ability effects for 9 X 9 diallel analysis of resistance to CAD

	Combined Environment		2003		2004	
Clones	LSM*	GCA	LSM	GCA	LSM	GCA
I30001	9.25	0.632	10.00	0.874	8.50	3.080
I30555	20.50	4.680**	20.50	6.026**	20.50	5.270**
I30572	21.25	0.017	20.00	1.848	22.50	1.764
I60142	13.75	-0.580	22.50	0.650	7.00	-2.520**
I63397	4.75	-3.200**	0.50	-3.438**	9.00	-2.504**
I90257	18.75	-0.680	12.00	-1.086	25.50	1.960
I4(2)1425	16.00	-2.670**	17.00	-2.570**	15.00	-0.504
TME 8	14.00	-2.890**	9.00	-4.082**	19.00	-2.280**
TME 9	11.00	-0.140	13.00	-2.750**	9.00	-0.504
SE (gi-gj)		1.983		1.840		1.672

*, ** Significantly different from zero at 0.05 and 0.01 probability levels, respectively
*LSM = least square means

to Singh and Chaudary [13]. Pearson correlations using line and top cross means were calculated to compare line and top cross.

RESULTS AND DISCUSSION

The analysis of variance for 9 X 9 diallel involving seven improved cassava clones (I30001, I30555, I30572, I60142 and I90257) and two landraces (TME-8 and TME-9) is presented in Table 1. There were variations ($p<0.05$) among the environments and genotypes in the combined analysis for CAD canker counts. Moreover, the contrast parent X crosses (the test for average heterosis) was significant for both the combined and individual environment. The Griffing analysis of variance for the crosses revealed significant GCA, SCA, maternal and specific reciprocal effects in both the combined and individual environments. The Crosses X E, GCA X E, SCA X E, REC X E and M X E effects were also significant for CAD canker counts. The maternal effects among the parents were significant just as the specific reciprocal effects among their crosses were also significant $p<0.01$. The genetic ratio, additive variance to total variance for the diallel analysis was 0.48, 0.41 and 0.43 for year 2003, 2004 and the combined environments, respectively. There were significant ($p<0.05$) relationships between the 9 parents performance per se and their GCA effects in individual environment and combined environments. ($r = 0.55^{**}$ for 2004, $r = 0.67^{**}$ and $r = 0.41^{**}$ for combined environment). However, the line performances of the parents were not significantly correlated to their mean top cross performance ($r = 0.04$ for 2003, $r = 0.15$ for 2004 and .0.29 in the combined environments. GCA and SCA sums of squares accounted for 43.44 and 56.56% of variation among crosses in the combined year, respectively. GCA accounted for 58.22 and 61.04% in year 2003 and 2004, respectively, while the SCA accounted for 41.84 and 38.95% variations among the crosses in year 2003 and 2004, respectively.

The estimates of GCA effects of each parent for total number of cankers/plant on cassava stems are presented in Table 2. Only negative values indicated contributions

Table 3: Estimates of specific combining ability effects and reciprocal effects for 9 X 9 diallel analysis of resistance to CAD

	Combined environment		2003		2004		
Crosses	LSM	SCA	LSM	SCA	LSM	SCA	Effects
I3000I X I30555	16.00	-3.53*	23.5	-5.28**	8.50	-3.07**	SCA
I3000I X I30572	10.50	-6.56**	19.0	-11.62**	4.00	-2.49*	SCA
I3000I X I60142	15.25	10.80**	22.0	12.47**	8.50	4.58	SCA
I3000I X I63397	9.50	2.94	9.0	-4.47**	10.00	4.25	SCA
I3000I X I90257	8.50	-0.35	15.0	-2.09*	2.00	-4.30**	SCA
I3000I X I4(2)1425	11.00	2.15	12.0	-1.44	10.00	4.24	SCA
I3000I X TME-8	4.75	-3.84**	0.5	-9.31**	9.00	-3.71**	SCA
I3000I X TME-9	14.25	1.07	22.0	-6.87**	6.50	0.74	SCA
I30555 X I30572	12.25	-3.51**	21.0	-5.81**	3.50	-3.23**	SCA
I30555 X I60142	16.25	2.11	25.0	-5.68**	7.50	0.09	SCA
I30555 X I63397	14.75	2.46	22.5	4.16	6.00	-0.70	SCA
I30555 X I90257	20.00	0.71	36.5	4.16	3.50	-3.66**	SCA
I30555 X I4(2)1425	12.50	-0.73	25.0	3.54	0.50	-1.21	SCA
I30555 X TME-8	6.63	-2.85**	13.2	-10.31**	0.50	-2.41	SCA
I30555 X TME-9	10.00	-0.87	18.5	-8.12**	1.50	-1.58	SCA
I30572 X I60142	7.25	-5.60**	7.0	-14.43**	7.50	-2.86*	SCA
I30572 X I63397	10.00	3.27	19.5	-1.09	0.50	-0.65	SCA
I30572 X I90257	21.25	0.50	36.5	-0.72	6.00	6.60**	SCA
I30572 X I4(2)1425	10.00	3.24	14.0	-8.31**	0.50	0.09	SCA
I30572 X TME-8	21.25	7.14	18.5	8.57	0.50	7.30**	SCA
I30572 X TME-9	7.25	0.67	17.5	-5.99**	0.50	-1.40	SCA
I60142 X I63397	0.50	-4.24**	0.5	-3.97*	0.50	-0.20	SCA
I60142 X I90257	0.50	-5.93**	0.5	-13.84**	0.50	-1.40	SCA
I60142 X I4(2)1425	0.50	-4.82**	0.5	-12.43**	0.50	-1.03	SCA
I60142 X TME-8	1.25	-3.58**	2.0	-2.06	0.50	-0.90	SCA
I60142 X TME-9	9.25	2.37	18.0	0.13	0.50	0.79	SCA
I63397 X I90257	8.25	5.07	11.5	1.97	6.00	4.62**	SCA
I63397 X I4(2)1425	1.25	-3.55**	2.0	-6.62**	0.50	-3.05**	SCA
I63397 X TME-8	2.25	-4.58**	4.0	-7.25**	0.50	-4.51**	SCA
I63397 X TME-9	16.25	5.39	26.5	3.94	6.00	-1.06	SCA
I90257 X I4(2)1425	4.00	-1.56	7.0	-9.04**	1.00	0.67	SCA
I90257 X TME-8	18.50	2.54	24.0	0.13	12.50	-3.53**	SCA
I90257 X TME-9	1.00	-4.12**	1.5	-8.68**	0.50	6.87**	SCA
I4(2)1425 X TME-8	0.75	-0.09	1.0	-4.72**	0.50	-1.83	SCA
I4(2)1425 X TME-9	12.75	-4.98**	21.0	4.47**	4.50	-4.82**	SCA
T0ME-8 X TME-9	7.50	-2.71*	14.5	-3.41**	0.50	-3.33**	SCA
I30555 X I30001	9.50	1.50	17.0	3.25	2.00	0.50	Recip.
I30572 X I30001	5.25	2.50	10.0	4.50	0.50	1.00	Recip.
I30572 X I30555	14.50	1.88	25.0	-2.22	4.00	3.25	Recip.
I60142 X 30001	22.00	-3.75	42.0	-10.00	2.00	1.75	Recip.
I60142 X I30555	10.75	1.38	21.0	2.40	0.50	0.75	Recip.
I60142 X I30572	6.00	2.25	6.0	0.75	6.00	0.50	Recip.
I63397 X 30001	6.25	0.50	12.0	-1.50	0.50	2.00	Recip.
I63397 X I30555	4.50	2.25	7.0	7.51	2.00	3.75	Recip.
I63397 X I30572	6.00	0.85	11.0	4.25	1.00	2.50	Recip.
I63397 X I60142	11.25	-11.50**	21.5	-10.75**	1.00	-11.50**	Recip.
I90257 X 30001	8.25	0.25	16.0	0.55	0.50	0.50	Recip.
I90257 X I30555	9.75	6.00	18.5	9.07	1.00	3.00	Recip.
I90257 X I30572	1.75	0.50	0.5	18.25	3.00	-0.25	Recip.
I90257 X I60142	5.00	0.75	7.0	-3.54	3.00	0.50	Recip.
I90257 X I63397	13.75	-2.63	18.0	3.25	9.50	1.75	Recip.
I4(2)1425 X 30001	11.50	-2.50	17.0	-2.75	6.00	2.00	Recip.
I4(2)1425 X I30555	14.25	0.13	25.0	0.50	3.50	0.25	Recip.
I4(2)1425 X I30572	3.75	3.00	4.0	5.00	4.50	1.25	Recip.

Table 3: Continue

	Combined environment		2003		2004		
Crosses	LSM	SCA	LSM	SCA	LSM	SCA	Effects
I4(2)1425 X I60142	4.75	9.00	6.5	-3.25	0.50	0.50	Recip.
I4(2)1425 X I63397	4.75	1.38	7.0	-2.54	2.50	1.25	Recip.
I4(2)1425 X I90257	5.15	0.75	3.0	2.07	11.30	2.00	Recip.
TME-8 X 30001	5.25	-5.38**	9.5	-4.75**	1.00	-5.00**	Recip.
TME-8 X I30555	5.25	2.25	6.0	3.52	4.50	2.25	Recip.
TME-8 X I30572	29.50	-0.25	29.5	-5.53	29.50	-1.75	Recip.
TME-8 X I60142	11.00	-8.88**	21.5	-9.75**	0.50	6.50**	Recip.
TME-8 X I63397	0.50	0.87	0.5	2.00	0.50	0.50	Recip.
TME-8 X I90257	2.25	3.37	0.5	12.25	4.00	-2.00	Recip.
TME-8 X I4(2)1425	6.75	1.00	10.0	4.51	3.50	-1.50	Recip.
TME-9 X 30001	4.25	6.25	8.0	7.02	0.50	4.25	Recip.
TME-9 X I30555	12.00	0.88	20.5	0.51	3.50	-1.75	Recip.
TME-9 X I30572	5.75	4.50	17.0	0.25	7.00	3.25	Recip.
TME-9 X I60142	14.25	-14.00**	25.5	-3.75	3.00	-1.50	Recip.
TME-9 X I63397	8.50	4.13	15.5	5.52	0.50	2.75	Recip.
TME-9 X I90257	16.00	1.07	20.5	8.50	11.50	0.50	Recip.
TME-9 X I4(2)1425	12.25	0.13	24.0	-1.54	0.50	2.25	Recip.
TME-9 X TME-8	5.75	3.13	11.0	1.75	0.55	0.50	Recip.
SE (sii)		3.96		5.29		3.76	
SE (sij)		1.69		2.22		1.59	
SE (sii-sjj)		5.01		6.57		4.65	
SE (ii-skj)		5.90		7.85		5.55	
SE (ij-skj		5.69		7.45		4.96	

*, ** Significantly different from zero at 0.05 and 0.01 probability levels, respectively
*LSM= least Square means

towards resistance, while positive significant values suggest a contribution towards susceptibility. The resistant lines I63397 had significant negative GCA effects in both Ibadan environments and in the combined environment showing its capacity to transmit resistance. The two landraces used in this study TME-8 and TME-9, which was susceptible to CAD, exhibited negative GCA in year 2003 and 2004 and across the environments. However, the susceptible improved clone I30555 had significant and positive GCA in all the environments. Susceptible improved clone I90257 had a non-significant negative GCA effect across the environments. The moderately susceptible improved clone I30001 had significant and positive GCA effects in the Ibadan 2004 environments. The highly susceptible improved clone I30572 had significant negative GCA.

The estimates of least square means, SCA and reciprocal effects of the diallel crosses is presented in Table 3. The crosses and their reciprocals manifested varying degree of resistance to CAD in the different environments. The crosses I60142 X I63397, I60142 X I90257, I60142 X I4(2)1425, I60142 X TME-8, I90257 X TME-9, I4(2)1425 X TME-8 were highly resistant across the environment and manifested significant and negative SCA effects. Crosses I30001 X TME-8, I63397 X I4(2)1425, I63397 X TME-8 and TME-8 X TME-9 (from two susceptible parents) were also resistant to CAD and had negative SCA effects across environments. Negative specific reciprocal effects for resistance to CAD were significant for crosses TME-8 X I60142, TME-8 X I63397, which were reciprocal crosses of highly resistant crosses with significant negative SCA effects. The moderately resistant crosses I30555 X TME-8 and I30555 X TME-9 also exhibited significant negative SCA across the environment. The susceptible crosses I30001 X I30555, I30001 X I30572 and I30555 X I30572 also had significant negative SCA effects. The susceptible cross I30001 X I60142 was detected to have significant positive SCA effects.

The significant genotypes X environment interaction observed in the study are an indication of lack of stability of across environments in development of CAD symptoms. This suggests that parents including the crosses must be evaluated in more than one single environment in order to obtain precise genetic information required. The general combining ability (GCA) and the specific combining ability (SCA) were found to be relatively important in determining progeny performance. The non-predominance of neither GCA nor SCA was further reflected by non-significant correlation between

the parental means and their GCA effects, which indicates that progeny performance cannot be determine from parental performance per se. The significant female by male interaction also confirms the presence of non-additive components in the resistance of crosses to CAD. The ratio of additive variance to total genetic variance in a population is an indication of relative importance of both GCA and SCA in predicting progeny performance in resistance of cassava to CAD. The closer this ratio is to one the greater the chances of predicting progeny performance based on GCA [14, 15].

The significance of the contrast, parent vs crosses justifying the separation of parents and crosses before the diallel analysis was done. The GCA and SCA sum of squares accounted for 43.44 and 56.56%, respectively of the variation among the crosses. This demonstrated that both additive and non-additives gene effects are also important in determining the expression of resistance to CAD in cassava even though the SCA contributed more.

The negative values of GCA effects of parental lines indicate a contribution towards resistance while positive values represent contribution towards susceptibility. The moderately resistant improved clone I63397, moderately susceptible improved I4(2)1425 and the susceptible local variety TME-1 had significant and negative GCA effects in the diallel analysis showing their ability to transmit resistance. Among the susceptible lines, the high capability of I30572 to transmit susceptibility is notorious. From this findings the magnitude and sign of GCA effects of each parent is not generally in agreement with their individual performance per se. This indicated that initial selection of parents for hybrid combination might not largely be based on the disease reaction. The significance of maternal and reciprocal effects suggested that the variation observed in this experiment was not only due to direct genetic effects. Maternal effects originate from differences in cytoplasm usually DNA in replicating organelles such as mitochondria, or from differences in maternal environment provided to the developing embryos [17]. Genotype I30572 had a high significant effect towards susceptibility. Using this line as female parent in the hybrid combination will not allow the expression of resistance governed by nuclear genes. The significant of parents' means of squares in the diallel analysis showed that diverse variability occurred among the parents suggesting that African landraces and IITA improved germplasm could be a source of resistance to CAD.

The present study report has significance implications for cassava breeding programs that seek to incorporate resistance to CAD. This is because in the diallel analysis, the additive effects as well as non-additive genetic effects are desirable for resistance to CAD. Hence the progeny performance may not be based on the parent performance per se. This investigation emphasized the need to screen parents and crosses before their use in breeding suggesting that combining ability analysis based on progeny test data is useful in cassava breeding programme. These conclusions are in agreements with the findings of Guar *et al.* [18] and Sharma *et al.* [19], who claimed that parents with high GCA effects did not necessarily produced hybrids with high SCA, but in contrast with the work of Mohammed and Sultan [20].

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Phosphorus Level Affects Brown Blotch Disease, Development and Yield of Cowpea

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Abstract: Application of Phosphorus in adequate concentrations could directly or indirectly reduce disease incidence and severity of cowpea brown blotch and improve the forage and grain yield. A field experiment was conducted to investigate the effects of level of phosphorus application on Brown Blotch disease of cowpea (*Vigna unguiculata* [L.] Walp), caused by *Colletotrichum capsici* during 2003, 2004 and 2005 planting seasons. Application of phosphorus was at 30, 60, 90 and 120 kg ha^{-1} of P_2O_5. As P level increased numbers of petioles, pods, nodules, seed/pod, leaf area and yield significantly increased. Disease incidence and severity of Brown Blotch were significantly reduced at 90 and 120 kg ha^{-1} of P_2O_5 was not affected irrespective of the method of application. Application method did not affect yield. Reduction in brown blotch disease at higher levels of P was recorded.

Key words: Cowpea % disease % methods % phosphorus % rate

INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp) is an important grain legume in drier regions and marginal areas of the tropics and subtropics. It is particularly important in West Africa with over 9.3 million metric tons of annual production [1]. The grain is a good source of human protein, while the haulms are valuable source of livestock protein [2]. Diseases of cowpea, induced by species of pathogens, constitute one of the most important constraints to profitable cowpea production wherever it is cultivated. Among the fungal diseases, brown blotch, caused by *Colletotrichum capsici*, is the most devastating with yield loss between 46 and 85% in Nigeria [3, 4].

Phosphorus fertilizer application has been observed to reduce rice blast disease [5]. He stated that repeated application of phosphorus fertilizers delays the onset and lessens the severity of take-all disease of barley just as potassium application reduces the disease incidence in many cases probably by increasing phenolics synthesis in plants Calcium application also reduces bean root rot caused by *Rhizoctonia solani* probably by altering pectin metabolism of the host [5]. Therefore, application of mineral elements in adequate concentrations could directly or indirectly reduce disease incidence and severity of crops.

Phosphorus, although not required in large quantities is critical to cowpea yield because of its multiple effects on nutrition and nitrogen fixation [6]. It also influences the content of other nutrients in cowpea leaves [7] and seed [8]. Agboola and Obigbesan [9] reported that P concentration in plant was highest with the use of single super phosphate, as opposed to rock phosphate. The present study was undertaken to determine effects of level and method of application of phosphorus on development, yield and severity of brown blotch disease in cowpea.

MATERIALS AND METHODS

Field operation: Brown blotch infected seeds of Ife brown variety of cowpea were collected from seed stored at the Institute of Agricultural and Training, Ibadan. Nigeria. Field experiments were conducted during 2003, 2004 and 2005 at the Institute of Agricultural and Training. The site is located at Latitude 7°31'N and longitude 3°45'E and 210 m above sea level in the forest-savanna transition agro-ecological zone of Nigeria. The field was laid out in a Randomized Complete Block Design. The treatments consisted of four levels phosphorus (0, 30, 60, 90 and 120 kg ha^{-1} of P_2O_5) and two methods of application (at planting or as a foliar spray).

Three years before the experiment started, the plot had been cropped continuously to maize with no fertilizer. The physicochemical properties of the field were analyzed before cultivation in each of the three years, with P level determined by the procedure of Leverty [10] (Table 1). The land was plowed and harrowed twice. There were 27 plots (nine treatments replicated three times) of 25 m^2

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Table 1: Mean values of analyzed physicochemical properties of the soil of the experimental site at Ibadan, Nigeria in years 2003, 2004 and 2005

Soil characteristics	0-15 cm soil depth
Percent clay	5.2
Percent silt	9.8
Percent sand	85.0
Soil Texture	Sand loam
pH (H_2O)	5.500
Organic carbon (%)	0.063
Total nitrogen (%)	0.070
Available phosphorus mg kg^{-1}	0.870
CEC (meq/100 g soil)	3.390
Ex changeable bases (meq/100 g soil)	
Ca^{2+}	2.040
Mg^{2+}	0.500
K^{+}	0.140
Na^{+}	0.350

each, with 1 m paths across the rows and along the rows. Four seed/hole were planted on 4 Sept. in each year were planted at a spacing of 75 X 30 cm and thinned two-weeks later to two plants/hole. At planting fertilizer was applied to the side of the cowpea hill and foliar application was done four weeks after planting. Weeds were controlled with a mix of Gramozone® (paraquat dichloride: Jubaili Agrotect Nigeria Limited) and Galex 500E® (metolachor + metobromuron) (Novartis Nigeria Limited, Mushin, Lagos, Nigeria) as a pre-emergent treatment at 2.5 L ha^{-1} with two hoe weeding at three and six weeks after planting (WAP).The herbicides were applied at rate of 3 L ha^{-1} each using the Knapsack sprayer. The interior four of seven rows per plot were used for data collection. The insecticide Nuvacron® (Monocrotophos: Afcott Nigeria Plc, Lagos, Nigeria) at the rate of 2 L ha^{-1} using the Knapsack sprayer.

Numbers of petioles were counted and leaf area measured at 50% pod formation, using a destructive procedure from the plants in the rows next to the border rows after the four interior rows, while numbers of pods, nodules, seed/pod, 100-seed weight and yield were assessed at maturity. Disease incidence was calculated from the number of infected plants in the population. The severity of brown blotch disease on pods on all plants in the four interior rows was determined using the visual assessment scale according to Alabi [3] and modified: 0: no symptoms, 1: up to 20%; 2: 1-40%; 3: 41-60%; 4: 61-80% and 5: over 80% pods covered with brown blotch.

Statistical analysis: The data were analyzed using the general linear model (GLM) Statistical Analysis System (SAS Inc., Cary, NC) at the 5% probability and means separated using the Fishers Protected LSD test.

RESULTS AND DISCUSSION

The soil was very low in available P (0.87 mg kg^{-1}). Table 2 shows the analysis of variance of the effect of the different levels and method of application of P on yield parameters and brown blotch disease of cowpea. The results show that significant differences occurred among the treatments irrespective of the parameters ($p<0.05$). The influence of the environment was significant only on the number of nodules per plant (Table 1). The number of petioles per plant (Table 2) as well as the number of pod per plant (Table 3) was not significantly different among the three growing seasons irrespective of the level and method of application. However, the environment significant influence the number of nodules when plants

Table 2: ANOVA table of the influence of levels and method of P application on yield components and brown blotch disease of Brown of cowpea

	Per plant						Disease		
Sources	Petioles	Pods	Nodules	Seed/ pod	1000 seed wt.	Leaf area	Incidence	Severity	Yield
Application									
method (A)	0.20	6.12*	4.56**	2.54	3.42	0.99	29.22*	1.98	17.23
P rate (P)	3.44**	13.22**	1.10	10.23**	6.56*	3.41*	52.35**	5.25**	65.33**
Year (Y)	0.43	0.89	0.82	2.58	3.33	1.20	16.25	1.88	17.52
Interaction									
A X P	2.33**	7.18*	13.77*	29.43**	3.86**	68.92**	18.16*	1.78**	47.98*
A X Y	0.01	0.05	0.55	2.75	1.45	1.47	3.89	0.36	6.20
P X Y	0.13	1.03	0.96	2.66	4.33	1.39	17.70	0.25	18.71
A X P X Y	0.19	0.71	0.88	0.86	1.93	0.05	6.90	2.05	13.25
Error	0.30	0.70	0.66	2.33	2.78	0.89	15.43	1.52	16.48

*, ** significant at $p < 0.05$ or $p < 0.01$, ANOVA

Table 3: Effect of levels and method of P application on yield components and brown blotch disease of Ife-Brown variety of cowpea

Application method	Phosphorus level (kg ha^{-1})	Petioles/ plant	Pod/ plant	Nodule/ plant	Seed/pod	100-seed wt.	Leaf area	Disease incidence	Disease severity	Yield (kg ha^{-1})
Broadcasting										
	30	29.22[a]	50.23d	25.89d	12.82d	102.3b	20.56[a]	66.33b	3.50b	993.85b
	60	24.78[a]	60.32c	36.00c	14.23bc	103.9b	24.11[a]	26.11de	2.78b	1454.58a
	90	26.67[a]	69.67a	42.67b	16.78a	128.4a	26.33[a]	24.89de	1.00c	1450.96a
	120	27.44[a]	75.12a	46.78a	16.88a	129.9a	27.67[a]	21.67cd	1.00c	1482.11a
Foliage Spray										
	30	27.89[a]	50.87d	26.33d	15.22b	105.4b	23.89[a]	48.00c	2.00c	983.07b
	60	24.44[a]	58.22b	39.55c	15.34b	120.8a	26.88[a]	30.33e	2.00c	1431.96a
	90	26.56[a]	69.00a	42.21b	17.12a	130.5a	26.77[a]	29.11d	1.00b	1462.50a
	120	32.66[a]	73.78a	47.56a	17.22a	137.1a	26.78[a]	25.44de	1.00b	1498.08a
control	0	12.44[b]	25.22e	19.44e	12.56d	85.5c	18.88[b]	80.11a	4.2a	647.74c

Values followed by the same letters are not significantly different Fishers Protected LSD test ($p<0.05$)

were sprayed with 30 kg haG^{1} and 120 kg haG^{1} with both application methods. There were also no significant variations in the effect of the phosphorus fertilizer among the three growing seasons on the number of seeds per pod leaf area (cm^{3}) 100-seed weight and yield kg haG^{1}. The application method by phosphorus rate interaction (A x P) were significant for all the growth parameters ($p<0.05$) just as it influenced significantly ($p<0.05$) the incidence and severity of brown blotch of cowpea. The application method by year interaction (A x Y) just as phosphorus rate by year interaction was not significant for all the parameters as well as for disease incidence and severity (Table 2). The results in Table 2 also showed that the application by phosphorus rate by year interactions (A x P x Y) was not significant The results in Table 3 show that significant differences did not occur among the levels P irrespective of the method of application on the number of petioles. However, the number of petioles in these treatments was significantly higher than in the control. The maximum number of petioles 32.66 was recorded when the plant was sprayed with 120 kg haG^{1} of P. Application of higher doses of P significantly increased the number of pods/plants and nodules/plant irrespective of method of application. The highest number of pods (75.12) was obtained when the crop received 120 kg haG^{1} of P fertilizer applied basally while, the maximum number of nodules of 47.56 was recorded when the same quantity of P was sprayed on cowpea. Application of higher doses P increased the leaf area compared to the control but all the treatment were statistically at par with each other. The number of seeds per pod increase significantly with increase in the levels of P fertilizer. There were however, no significant differences in the number of seed per pod between the control and basal application of 30 kg haG^{1} of *P*. *Foliar* spray of P at 120 ka haG^{1} gave the highest number of seed per pod (17.22), while the control experiment gave the lowest seed/pod of 12.56. The data in Table 3 revealed a significant increase in 1000-grain weight with increase in the concentration of P irrespective of method of application. However, foliar application of 120 kg haG^{1} of P gave the maximum 1000-grain weight (137.10 g), while the control gave the lowest 1000-grain weight of 85.5 g. The results indicated that all the fertilizer treatments gave significantly higher yield as compared to control. Table 3 also showed that P application significantly increased the grain yield of Ife-Brown cowpea variety compared with the recommended 30 kg haG^{1} and the control. However, among the fertilizer treatments, foliar application of 120 kg haG^{1} of P produced the highest grain yield (1493.07 kg haG^{1}). The results in Table 3 also show that application of P at 90 and 120 kg haG^{1} irrespective of method of application reduced the incidence and severity of brown blotch disease significantly. The highest disease incidence and severity were observed with control.

The significant response of cowpea variety to P application in terms of growth parameters, grain yield and reduction in the incidence and severity of brown blotch disease is an indication that P is an important nutrient element influencing the performance of cowpea plant on the field. Tenebe *et al*. [11], Ankomah *et al.* [12], just as Okeleye and Okelana [13], observed significant increase in nodulation, grain yield, total dry matter, numbers of flower, pods and seed per plant for cowpea varieties in response to P application. The increase in nodulation observed in this study contradicts the findings of Kolawole *et al*. [14], which reported a decrease in number of nodules due to increase in P application, but agrees with the results of Luse *et al.* [15], Agboola and Obisesan [9] and Ankomah *et al*. [12]. The observed increase in cowpea grain yield due to increase in level of P in the present study is in consonance with the results reported by Tenebe *et al*. [11], Ankomah *et al.* [12] and Kolawole *et al*. [14], but disagrees with the results obtained by Agboola and Obigbesan [9] and Osiname [16], who did not observe significant effect of on the yield of cowpea

with increased levels of P at Ibadan, but rather enhanced nodulation and P content of leaf and stem. The results of the present study also show that application of P significantly reduced the incidence and severity of brown blotch disease of cowpea. The response of Ife-Brown variety of cowpea to this disease could be as a result of enhanced root growth and development, consequently improved nutrient uptake for vigorous vegetative growth that could have resulted in disease escape. The results aggress with the report of Tenebe *et al*. [11] that increase in height and vegetative growth of cowpea lines with an increase in P application. This investigation also observed that significant differences did not occur between the two application methods except for the fact that 120 kg ha^{-1} P applied by foliar spray gave the highest yield. However, it was observed that cowpea plants that received foliar spray of P had their leaves more greener (dark green) and healthier those with basal application Moreover, the time and amount of energy expended on foliar application is less than in application. Thus, foliar application of 120 kg ha^{-1} of phosphorus from SSP ensures the high yield and reduction in incidence and severity brown blotch disease of Ife-Brown variety of cowpea, but 60 kg ha^{-1} of phosphorus basal application gave an optimal yield of the cowpea variety.

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Line x Tester Analysis for Resistance to Cassava Anthracnose Disease

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Abstract: Thirteen cassava (*Manihot esculenta)* varieties which includes four IITA Improved used as lines and seven Landraces used as testers with various level of resistance to *Colletotrichum gloeosporioides* f. sp. *manihotis*, were crossed in a Line X tester design to determine the general (GCA) and specific (SCA) combining abilities relative to the inheritance. The Parents and the 36 F1 hybrids were evaluated in year 2003 and 2004 on an infected field. The variances due to SCA and GCA showed that both additive and non-additive, possibly epistatic gene actions are important. Majority of the crosses between the resistance sources and the susceptible lines showed intermediate reactions and various degrees of partial dominance for canker development in cassava plants. The most resistance IITA improved variety I63397, had the highest negative GCA effect for resistance among the lines. The moderately resistance TME-8 had largest significant negative GCA among the landraces. Most the crosses involving I63397 and TME-8 had significantly high negative SCA effects. The contribution of these parents to heterosis of their hybrids will be towards reduction of disease symptoms. This suggests the importance of both the additive and non-additive in the development of resistance to cassava anthracnose disease. Therefore recurrent selection with progeny evaluation is advocated for breeding for resistance to the disease.

Key words: *Colletotrichum gloeosporioides* f. sp. *manihotis* % combining ability % heterosis % line xtester analysis % *Manihot esculenta*

INTRODUCTION

Cassava Anthracnose Disease (CAD) cause by a fungus *Colletotrichum gloeosporioides* f. sp. *manihotis* has been reported to be an epidemic disease characterized by cankers of stems, branches, fruits, leaf spots and die-backs [1-3]. In some susceptible cultivars, blight and dramatic wilt of infected leaves may occur [1]. On young cassava stems, CAD is characterized by oval, pale brown, shallow depressions which could lead to petiole epinasty, necrosis, wilting and defoliation [4-6]. Owuneme [7] as well as Van der Bruggen [8] observed that infection on older stems usually occur as round and stringy lesions that develop into deep cankers followed by stem deformation, causing the stem to become brittle and easy to break by wind action. The geographical distribution of CAD is world-wide as observed by Lozano *et al*., [9]. In Africa, CAD is presently considered to be of major importance in terms of its potential for causing stem damage in cassava. Muyolo [5] observed that 90% of local cassava in Zaire were severely affected by CAD just as Makambila [10] reported that more than 80% of cassava plants in Congo showed CAD symptoms. Ikotun and Hahn [11] also showed that the quality of plantable materials and germination of cuttings are reduced by CAD infection in Nigeria. They also observed that large cankered cassava stems are weak in the field and liable to breakage during storms. It has also been reported that, just as infected cuttings is a source of inoculum, large cankers caused by CAD serve as entry point for other pathogens of cassava [12-14].

However, due to the synergetic relationship among the Africa Cassava Mosaic Virus Disease (ACMV), Cassava Bacterial Blight (CBB), Cassava Anthracnose Disease (CAD) and Root and Tuber rots, makes breeding for resistance appear to be the most efficient and economical means for the control of CAD. Variety improvement depends greatly on screening parental lines to be used for hybridization programme. Information regarding the relative importance of General and specific combining ability estimates and type of gene action are very important for the improvement of cassava plant. The information obtained could be an essential tool for the cassava breeders in selection of better parental combinations in the breeding programme. Successful improvements of resistance to ACMV and CBB have been reported in breeding programs using half-sib family selection [15]. The desirable characteristics in F_1 half-sib

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plants are the results of both additive effects of genes and this last permanently through vegetative propagation unless mutation occurs [16]. Moreover, the mode of resistance could be quantitative in expression and polygenic in inheritance. However, no work has been done on the mode of inheritance of cassava to CAD under tropical climatic conditions. Therefore, the main aim of this study was to determine the relative importance of general and specific combining ability for resistance to CAD using the Line x Tester analysis.

MATERIALS AND METHODS

The experimental materials included International Institute of Tropical Agriculture, Ibadan, Nigeria (IITA) improved cassava clones (TMS I30001, I30555, I30572 and I63397) as females (lines) and African cassava landraces (TME 3, 4, 6, 7, 8, 9, 11 and 117) as males (tester). The seeds were developed by hand pollination in a Design II mating scheme at the IITA research field in Ubiaja, Edo State, Nigeria in 2001. In year 2002, the seedlings from these crosses were established at IITA experimental fields in Ibadan. The progenies and their parents were evaluated under rain fed conditions in year 2003 and 2004 growing seasons at the IITA's research farms in Ibadan, Nigeria, for their reactions to CAD. The experimental design for the study was a Randomized Complete Block Design with three replications. Each plot consists of a minimum of 40 plants spaced 0.5 m apart in rows (ridges 30 cm high and 10 m long) spaced 1 m apart giving a plant population of 20,000 plants per hectare. No fertiliser or herbicide was applied during the course of the experiments and hand weeding was done when necessary.

The reactions to CAD of the F_1 hybrids and their parents were evaluated at 12 months after planting. Individual plant was examined for symptom severity using the parameters and method adopted by Ikotun and Hahn [11].

The general linear model (GLM) procedure in SAS which uses the method of least squares was used for the analyses of variance [17]. Mean squares were calculated from type III sum of squares. Genotypes were partitioned into the variation due to lines (parents and crosses) and checks. The parents were further partitioned into female, male, resistant and susceptible parents.

Freedom orthogonal constrast between parents and crosses was used to test the presence of average heterosis. Orthogonal contrasts, female versus male parents and resisitant versus susceptible parents were also made to test for variation between the parents. Crosses were further analysed into variation due to the GCA (additive) effect of males. The GCA effect of females and variation due to the SCA (non-additive) effect of the male and female interaction. Separate analyses of variance was calculated on all entries including F_1 hybrids and parents for individual season. A combined analysis was also be performed to test the significance of the entries X seasons interaction. The linear model assumed are:

$$y_{ijk} = \mu+m_i+f_j+mfi+l_k+b_{l(k)}+ml_{ik}+fl_{jk}\,mfl_{ijkl}+0ijkl$$

(for the combined analysis)

$$y_{ijk} = \mu+mi+fj+mfi+bk+0ijk$$

(for the individual season analysis)

Where; y_{ijk} is the response of the kth observation in the ith environment of the plant; μ general mean; m_i the effect of ith male; fj was the effect of the jth female; mf_{ij} the interaction effect; b_k the effect of the kth year; $ml_{ik}$the effect of the jth male in the kth year; fl_{jk} effect of the ith female in the kth year; 0_{ijk} the error associated with each observation. All effects in the models were considered fixed [18].

RESULTS AND DISCUSSION

The analysis of variance for Line X Tester population is presented in Table 1. The results showed significant difference due to the environment and genotypes in each of the environment and across environments for CAD infection. Orthogonal contrast of Parents, female vs male were significant ($p<0.01$) in year 2003 & 2004 and across the environment. The female parents varied significantly across the environment and 2004 environment while the male parents varied significantly only in 2003 environment. Reactions of F_1 crosses to CAD symptoms severity were significant $p<0.01$ in year 20003 and 2004 and across the two environments. The GCA effects of the female was significant only in 2004 environment and across the environment, while the GCA effects of males was only significant in year 2004 but not across the environments. The GCA effects of the females contributed to the significant variation among the crosses. The parent vs cross contrast which is a test for heterosis was significant in the in the individual environment and across the environments. All the partitions; G x E, P x E, F x E, M x E, F x M x E and P vs C x E were significant indicating lack of stability of these effects across environments.

Table 1: Analysis of variance table for Line X Tester Mating Design for resistance to CAD

Source of variation			df	Combined	2003	2004
Environment (E)			1	15.46**		
Replicate (E)			2	0.44'	0.64'	0.24'
Genotype (G)			48	9.62**	9.10**	2.48**
	Parent (P)		12	14.88**	2.09**	1.82**
		Female (F)	3	6.43**	1.16'	1.13*
		Male (M)	8	1.37*	1.15'	1.57*
		F vs M	1	2.68**	5.56**	2.28'
	Crosses (C)		35	5.58**	11.60**	0.75*
		Female (F)	3	23.48**	11.64**	1.87*
		Male (M)	8	8.34**	2.98**	0.56'
		F X M	24	10.19**	7.79**	0.62'
	P vs C		1	6.73**	8.43**	2.85**
G X E			96	8.93**		
	P X E		24	9.86**		
	C X E		70	4.03**		
		F X E	7	9.55*		
		M X E	17	5.03**		
		F X M X E	48	11.01**		
	P vs C X E		1	76.96**		
Error Genotype			144	0.48'	0.65'	0.32'
Error Crosses			105	0.46'	0.63'	0.30'
GCA : SCA				0.54'	0.48'	0.57'

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

Table 2: GCA Effects for CAD of parents in 4X 9 Line X Tester Analysis Involving Four Lines (Female) and Nine Landraces (Males)

	Combined environment		2003		2004	
Genotypes	LSM	SCA	LSM	GCA	LSM	GCA
I30001	8.38	-0.18	7.23	1.55**	9.53	-0.86**
I30555	36.18	-0.33*	36.43	1.41**	35.92	0.45*
I30572	18.06	1.54*	15.27	0.67	20.85	0.39*
I63397	6.45	-1.04**	6.64	-0.32**	6.26	-0.22
SE	4.15	0.40	4.05	0.14	4.26	0.20
TME-117	9.00	2.47**	6.38	0.62	11.61	0.33*
TME-11	22.90	-0.69*	16.70	-2.02**	29.10	-0.54**
TME-12	10.87	-0.08	8.16	0.46	13.58	0.45*
TME-3	14.39	-0.37*	5.85	-0.02	22.93	-0.26*
TME-4	10.75	0.38	7.56	1.05	13.93	-0.22
TME-6	9.19	-0.54*	6.86	-1.76**	11.53	-0.56**
TME-7	6.52	-1.05**	9.29	-0.44**	3.75	-1.44**
TME-8	17.20	-0.46*	8.18	-0.01	26.23	0.94**
TME-9	20.06	-0.11	8.11	1.76	32.00	-0.75**
SE	3.66	0.32	2.92	0.18	4.28	0.24

*, ** Significantly different from zero at 0.05 and 0.01 probability levels respectively, * LSM = least Square means

The genetic ratios, additive variance to total genetic variance were 0.48, 0.77 and 0.61 for year 2003, 2004 and the combined environments, respectively.

The GCA effects and least square means of parental clones in each environment and across environment are presented in Table 2. A parent with a total number of cankers ranging from 0-5 was classified as resistance, those with 6-10 were classified as moderately resistance and those with mean total number of cankers greater than 10 were classified as susceptible. The susceptible female parents were I300555 and I30572 while the susceptible male parents were TME-3, TME-6 and TME-7. The IITA improve clone I63397 had significant negative GCA effects in all the environments indicating that it was a good general combiner. A moderately resistant parent I30001 had a non-significant negative GCA effect in the 2004 environment and in the combined environment. The moderately resistant TME-6 and TME-7 had a significant negative GCA effects in the two Ibadan environments. The moderately resistance TME-117 had positive GCA effects in all the two environments.

The present study provides information on the inheritance of CAD resistance in cassava based on Line X tester analysis involving parents with diverse origin and resistance to CAD. The significant genotypes X environment interaction observed in this

Table 3: Estimates of specific combining ability effects for CAD among the crosses in Line X Tester mating Scheme

	Combined Environment		2003		2004	
Crosses	LSM	SCA	LSM	SCA	LSM	SCA
I30555 X TME-117	5.16	-3.07**	6.32	-2.34**	4.00	-0.22
I30555 X TME-11	9.38	1.10**	10.83	-1.48**	7.92	-0.08
I30555 X TME-12	6.64	-0.50	9.95	-0.14	3.33	-1.31**
I30555 X TME-3	5.58	-0.94*	7.97	-0.89*	3.18	-0.65
I30555 X TME-4	9.41	-1.30**	10.72	-0.67	8.10	1.75**
I30555 X TME-6	10.42	-1.80**	12.71	-0.29	8.13	-1.70**
I30555 X TME-7	4.58	-1.18**	8.70	-0.96*	0.80	-1.73**
I30555 X TME-8	7.48	0.38	8.18	0.38	6.77	-0.73*
I30555 X TME-9	5.50	0.92*	8.11	-1.51**	2.88	-0.06
I30001 X TME-117	8.39	-1.35	12.78	0.77	4.00	-1.01**
I30001 X TME-11	8.79	1.13**	15.28	0.61	2.73	-0.82**
I30001 X TME-12	5.35	-1.48	6.55	-1.98**	4.14	-1.10**
I30001 X TME-3	6.35	1.30**	10.83	0.40	1.66	-1.61**
I30001 X TME-4	4.76	-0.15	8.31	-2.01**	1.20	-1.90**
I30001 X TME-6	9.87	1.98**	17.04	-1.73**	2.70	1.66**
I30001 X TME-7	3.76	-1.39**	6.91	-2.00**	0.60	-1.03**
I30001 X TME-8	2.27	-1.49**	4.31	-2.81**	0.22	-3.54**
I30001 X TME-9	6.69	-0.31	8.94	-2.31**	4.44	0.26
I30572 X TME-117	4.93	-1.47**	5.55	-2.01**	4.30	-0.56
I30572 X TME-11	3.23	0.54	6.13	-3.09**	0.33	-1.72**
I30572 X TME-12	7.76	1.67**	7.65	1.64**	7.86	-1.06**
I30572 X TME-3	4.00	-1.17**	3.65	-2.40**	4.54	0.13
I30572 X TME-4	4.49	0.54	3.51	-0.45	5.46	-0.53
I30572 X TME-6	6.38	-3.02**	7.11	-2.37**	5.64	-1.16
I30572 X TME-7	7.25	-0.39	9.50	-0.12	5.00	-1.47*
I30572 X TME-8	8.40	0.43	11.25	1.53**	5.44	0.63
I30572 X TME-9	9.25	-0.80**	12.94	-0.50	5.56	-0.60
I63397 X TME-117	7.34	-1.48**	8.07	-0.08	6.60	-0.93*
I63397 X TME-11	6.49	-0.14	11.75	-0.50*	1.22	-0.93*
I63397 X TME-12	5.14	-0.93*	5.46	-0.84*	4.82	-0.12
I63397 X TME-3	5.17	-0.41	6.41	-0.60*	5.00	0.70
I63397 X TME-4	2.50	-1.10**	5.00	-1.28**	0.00	-1.86**
I63397 X TME-6	5.83	-0.14	7.15	-2.85**	4.50	-0.39
I63397 X TME-7	0.88	-1.98**	1.75	-1.70**	0.00	-1.69**
I63397 X TME-8	10.64	3.16**	9.70	0.60*	11.57	2.78**
I63397 X TME-9	5.98	-0.76*	8.35	-0.25	3.61	-4.85**
SE	2.50	0.56	2.79	0.49	2.07	0.40

*, ** Significantly different from zero at 0.05 and 0.01 probability levels, respectively, *LSM= least Square means

design is an indication of lack of stability of across environments in development of CAD symptoms. This suggests that parents including the crosses must be evaluated in more one single environment in order to obtained precise genetic information required. The general combining ability (GCA) and the specific combining ability (SCA) were found to be relative important determining progeny performance in the Line X Tester mating designs. The non-predominance of neither GCA nor SCA was further reflected by non-significant correlation between the parental means and their GCA effects, which indicates that progeny performance cannot be determine from parental performance per se. The significant female by male interaction also confirms the presence of non-additive components in the resistance of crosses to CAD. The ratio of additive variance to total genetic variance in a population is an indication of relative importance of both GCA and SCA in predicting progeny performance in resistance of cassava to CAD. The closer this ratio is to one the greater the chances of predicting progeny performance based on GCA [18, 19].

In the Line X Tester analysis, the results also showed that the moderately resistant I63397 had significant and negative GCA, while the highly susceptible improved I30572 had positive negative GCA. This indicates that I63397 had the ability to transmit resistance while I30572 had the capability to transmit susceptibility. The Landraces TME-8 and the improved I63397 were good general combiners while I30572 was a very general combiner in both Ibadan environments.

Significant and negative SCA effects were desirable of resistance. A cross with significant and negative SCA implies that this cross was more resistance than average while a cross with positive SCA implies that this cross

Table 4: Mid parent heterosis (MPH) and high parent heterosis (HPH) among F1 crosses for resistance to CAD in Line X Tester analysis

	Combined Environment		2003		2004	
Crosses	MPH	HPH	MPH	HPH	MPH	HPH
HPHI30555 X TME-117	-23.24	19.57	87.66	76.76	-85.86	-71.35
I30555 X TME-11	-56.56	3.10	27.65	111.34	-62.15	-58.03
I30555 X TME-12	-57.43	-44.15	-14.93	-9.40	-64.18	-56.55
I30555 X TME-3	6.88	14.79	65.90	85.13	-87.08	-82.53
I30555 X TME-4	-20.78	-19.92	113.07	9.92	-89.76	-87.40
I30555 X TME-6	89.06	148.54	142.04	148.75	-74.35	-71.66
I30555 X TME-7	-13.07	-14.91	-16.34	-4.42	-90.96	-93.70
I30555 X TME-8	-69.36	53.16	-44.09	-40.38	-98.76	-97.69
I30555 X TME-9	-39.53	-16.23	22.47	23.37	-78.62	-53.41
I30001 X TME-117	-80.89	-70.95	-69.90	-94.00	-75.64	-72.78
I30001 X TME-11	-75.40	-67.81	-59.24	-35.15	-83.24	-65.54
I30001 X TME-12	-70.91	-46.27	-63.40	-21.94	-86.54	-75.48
I30001 X TME-3	-67.63	-20.04	-62.30	-36.23	-89.19	-86.12
I30001 X TME-4	-63.46	-4.56	-53.39	-41.71	-67.50	-41.85
I30001 X TME-6	-46.75	53.18	-41.27	-85.54	-65.75	-29.49
I30001 X TME-7	-69.83	-12.93	-61.94	-6.35	-95.98	-78.66
I30001 X TME-8	-76.76	-63.70	-53.92	27.26	-78.23	-74.19
I30001 X TME-9	-68.80	-46.52	-53.38	26.51	-91.52	-91.00
I30572 X TME-117	60.07	64.07	-48.75	-13.01	-98.41	-98.41
I30572 X TME-11	-76.83	-74.61	-61.53	-60.51	-73.50	-62.96
I30572 X TME-12	-10.16	7.45	-34.72	-6.25	-54.27	-41.90
I30572 X TME-3	-41.34	-11.43	-66.76	-40.00	-79.25	-80.19
I30572 X TME-4	-29.57	11.57	98.00	52.24	-68.60	-60.80
I30572 X TME-6	-46.75	20.97	-35.71	3.80	-65.16	-51.08
I30572 X TME-7	-45.91	9.76	-22.64	2.26	-59.35	33.33
I30572 X TME-8	-583	-34.71	-4.09	-37.53	-76.89	-73.90
I30572 X TME-9	-11.25	-36.88	9.65	59.56	-78.96	-73.33
I63397 X TME-117	-33.49	24.65	23.96	26.48	-93.09	-180.51
I63397 X TME-11	-54.40	1.08	68.00	-29.64	-26.17	5.43
I63397 X TME-12	-43.15	-12.40	-26.35	-17.77	-51.31	-23.00
I63397 X TME-3	-42.46	-30.23	2.56	9.57	-66.14	-20.13
I63397 X TME-4	32.09	-20.93	-29.57	-24.69	-100.00	-100.00
I63397 X TME-6	6.03	32.81	5.93	7.68	-49.43	-28.11
I63397 X TME-7	-60.11	-56.58	-78.04	-73.64	-100.00	-100.00
I63397 X TME-8	-34.18	20.00	30.90	46.08	-28.80	84.98
I63397 X TME-9	-31.02	13.79	13.14	25.75	-67.93	-42.33
AVERAGE	-36.33	-10.52	-8.07	-3.65	-42.33	-59.99

*, ** Significantly different from zero at 0.05 and 0.01 probability levels, respectively

was more susceptible than average. Several of the crosses in this study manifested significant and negative SCA. Most of the crosses involving TME-8 and I633397 had significant negative SCA. This indicates their tendency towards resistance.

The average mid- and high-parent heterosis among crosses were -8.07 and -3.64%, respectively for year 2003 and -75.05 and -59.99%, respectively for 2004 environment (Table 3). In the combined environment the average MPH and HPH among the crosses were -36.33 and -10.52%. These average MPH and HPH values indicated that the F1 were generally more resistance to CAD than their parents in both 2003 and 2004 environments.

The significant negative MPH and HPH for the combined environment ranged from -20.78 to -80.89% and from -19.92 to -67.81%. In the 2003 environment significant negative MPH and HPH ranged from -22.64 to -78.04% and from -34.69 to 94.00%, while in year 2004 it ranged from -26.17 to -100% for MPH and -20.13 to 18.00% for HPH. Twenty-one crosses (58.33%) had negative MPH and HPH in both Ibadan environments and in the combined environments, out of which 15 crosses showed significant heterosis (Table 4). The results also showed that all the crosses in year 2004 showed significant and negative MPH. All the crosses involving 30001 as female except I30001 X TME-7 and I30001 X TME-4 in 2003 environment had significant and negative (MPH and HPH) heterosis in both environments and in the combined environment. The most heterotic cross which contributed significantly to the average MPH and HPH in the 2003 and 2004 environments and in the combined environment was I633397 X TME-7.

The significant of parents means of squares in the Line X Tester analyses showed that diverse variability occurred among the parents suggesting that African landraces and IITA improved germplasm could be a source of resistance to CAD. Moreover, the significant and negative mid- am d high parent heterosis observed for CAD severity in many crosses confirmed that the landraces and IITA improved genotypes are good sources of resistance to the disease. The presences of significant contrast, parents' vs crosses generally indicated the presences of desired average mid- and high parent heterosis. However, heterosis was unstable due to the significance of the contrast P vs C X E which also indicates an absence of dominance genetic effect. The closeness of the estimated least squares means for mid parent values and various crosses confirmed polygenic inheritance and absence of dominance. No dominance is assumed if the mean of F_1 crosses is equal to that of mid parent [20]. The results implies that non-additive component detected by the significance of SCA effects in Line X Tester experiments may therefore be due to additive epistatic effects which causes heterosis. Hence exploitation of additive and non-additive components of genetic variance population breeding based on large scale crossing and recurrent selection with progeny testing is contemplated for improving the tuber yield in cassava.

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Evaluation of the Mineral Nutrients and Organic Food Contents of the Seeds of *Lablab purpureus, Leucaena leucocephala* and *Mucuna utilis* for Domestic Consumption and Industrial Utilization

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Abstract: Investigations were carried out on the mineral nutrients and organic food contents of seeds of three fodder *Lablab purpureus*, *Leucaena leucocephala* and *Mucuna utilis* for domestic consumption and industrial utilization. *Leucaena leucocephala* seeds contained the highest amount of lipids crude protein carbohydrates and ash contents. *Lablab purpureus* contained the lowest amount of lipid crude protein crude fibre and ash content. The seeds of *Mucuna utilis* has the highest fibre content. Seeds of *L. leucocephala* have highest mineral nutrient contents, which includes N, P, K, Ca, Mg, Mn, Fe, Cu and Zn. However the seeds of *L. purpureus* contained P, Cu and Mg in higher quantities than the seeds of *M. utilis* of the three seeds, *L. leucocephala* seeds contained the highest saponification value, least iodine and acid contents. Seeds of *L. leucocephala* were found to be good for consumption and the oil can be used industrially for soap making.

Key words: Mineral nutrient % organic food % domestic comsumption % forage % saponification

INTRODUCTION

Fodders are known as forages. These are crops grown primarily for feeding farm animals. Forages include *Lablab purpureus*, *Leucaena leucocephala* and *Mucuna utilis*. Growing animal legumes as manures for incorporation in the soil in rotation with other crops can have several beneficial effects. As a rule, the most important effect as assumed, is increase in the available nitrogen of the soil. Which is thought to form the rapid decomposition of buried plant materials [1]. This phenomenon will give rise to immediate recycling with a relatively low C:E ratio, which enables the microbes to rapidly fix nitrogen at higher rate [2]. Moreover, legumes are normally of better nutritive values than grasses because legumes have higher contents of protein, calcium, phosphorus and lower contents of fibres [3].

From present study, it is found out that research on forage legumes was negligible in the past. Hence, most works on this group of plants are recent especially in the 19th century. However, reasonable amount of research was carried out on forages in India [4]. These reseachers studied some varieties of Indian leguminous plants. Apart from being used as fodders, some of these legumes can serve as direct food for human consumption [5]. Other uses of these legumes, include their being used as pulp, lumber firewood and charcoal [6]. Some are used in the production of gum [7], while some could be used as warm expellers in contraceptives, abortion as well as in the making of necklaces and decoration of household items [8]. Literature shows that these seeds are of different origin, belonging to different sub-families of the family Fabaceae [9]. *Leucaena* originates from central America and Mexico [10] and is a member of the sub-family Mimosoidae, while *Mucuna* and *Lablab* belong to the sub-family papillionodae originating from Guinea and tropical Asia. It is likely that the fodders would contain organic food items and mineral nutrients which are not only useful to animals but to man as well. Hence, this investigation reports on the food and nutrient components of the seeds of *Lablab purpureus*, *Leucaena leucocephala* and *Mucuna utilis* for possible use in future as food for human consumption and industrial utilization.

MATERIALS AND METHODS

The seeds of *L. purpureus*, *Leucaena leucocephala* and *Mucuna utilis* were collected from Forest Research

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Institute of Nigeria (FRIN) in Ibadan. All the seeds sample wise were stored in sterile white polythene envelops and kept inside desicator to prevent attack by microorganisms.

Determination of food contents: The dry seeds of each sample were ground into powder form using pestle and mortar. The powder was sieved through a 0.002 mm wire mesh to obtain fine powdered forms. Each sample of the powdered seeds were kept in McCartney bottles and stored in the dessicator for analysis later.

Determination of the lipid content: The lipid content was determined using petroleum ether in soxhlet reflux extractor as described by Block [11].

Determination of carbohydrate contents: Four grammes of each of the powdered sample seeds were weighed into sterile filter paper and wrapped. Each sample was extracted with 100 mL of 80% ethanol for 12 h in a soxhlet reflux extractor. The extractions were then evaporated into dryness in a vacuum evaporator. The residues were then each dissolved in 5 mL of sterile distilled water for chromatography work using the method of Dubois *et al*., [12] and modified by Faparusi [13].

Determination of crude fibre content: The crude fibre content was determined using the Acid-base method of AOAC [14]. Two grammes of the powdered form of each seed sample was poured into measured sinister digesting thimbles of a tecator filler equipment. The thimbles were hooded after measuring the samples. Already boiled 30 mL Hcl solution was introduced into each of the thimbles through a funnel and allowed to digest for 30 min. Later 30 mL NaOH solution was introduced into each thimble and again allowed to digest for 30 min. The thimbles were washed with hot boiling distilled water. The thimbles were then removed from the hood and taken to the oven maintained at 100°C to dry before cooling in the desiccators. The thimbles were re-weighed and the difference between the final weight and the initial weight of the used thimbles was determined.

Determination of crude protein: Protein contents of the samples were determined using the method described by McKee [15], Osborne and Voogst [16].

Determination of saponification value: Two grammes of each oil sample was weighed into conical flasks 25 mL of alcoholic KOH solution was added. Reflux condensers were attached to each conical flask. The flasks were heated in boiling water for 1 h with frequent shaking. Ten milliliter of phenolphthalein (1%) solution was added to each solution in the conical flask. Each solution was titrated with hot 0.5 mL HCl to determine the saponification value. The experiments were replicated three times.

Determination of acid and iodine values: Two grammes each of the extracted oil were used for the determination of both the acid and iodine value using the methods described by AOAC [14] and Alabi *et al.*, [17].

Determination of mineral elements: The mineral elements were determined using the analytical method of determining mineral constituents of food products [18]. Samples obtained through ashing were used for this procedure which was the white fluffy mas. Five milliliter of concentrated hydrochloric acid was used to digest each ash content in a glass petridish. The mixture was transferred to 50 mL chemical flask using distilled water. Particles which cannot dissolve and would cause contamination were filtered off using Whatman's no. 1 filter paper in a funnel. The new filterate was made up to mark in readiness for mineral nutrient determination. The elements determined include Na, Ca, K, P, Mg, Mn, Fe, Cu and Zn. The determination was made using method described by Hack [18] standard reagents for the various elements to be determined were prepared. The series spectrophotometer was first warmed up for 30 min. Then, the standard reagent of the elements to be determined and distilled water were used to standardize the equipment. The samples contained in 10 mL cuvette was then introduced into the sample chamber where the samples were read and recorded.

RESULTS

The results show that *Leucaena leucocephala* seeds contain the highest amount of lipid, crude protein, carbohydrates and Ash contents. The values obtained were significantly higher ($p = 0.05$) than those obtained for *M. utilis* and *L. purpureus* seeds (Table 1). *L. purpureus* seeds contained low amount of lipid, crude protein, crude ash contents while seeds of *M. utilis* contained higher amount of crude fibre (Table 1). Seeds of *L. leucocephala* have the highest amount of mineral contents such as P, K, Ca, Mg, Na, Mn, Fe, Cu and Zn. The values obtained were significantly higher ($p = 0.05$) than those of *M. utilis* and *L. purpureus* (Table 2) *M. utilis* contained the highest amount of N (Table 2).

Table 1: Food analysis of *L. purpureus, L. leucocephala* and *M. utilis* seeds

Type of seeds used	Lipid content (%)	Crude protein content (%)	Carbohydrate content (%)	Crude fibre content	Ash content mg/100 g
Lablab purpureus	4.3±0.2c	1.14±0.22b	24.48±0.32c	10.5±0.80b	9.62±0.90c
Leucaena leucocephala	12.5±0.5a	8.40±0.15a	40.56±1.20a	4.33±0.26c	21.46±1.20a
Mucuna utilis	7.5±0.3b	1.42±0.23b	26.89±0.39b	13.96±1.00a	12.28±0.84b

Table 2: The mineral contents of seeds of *L. purpureus, L. leucocephala* and *M. utilis*

	Cations value mg/100 g					Non-ions value mg/100 g				
Types of seeds used	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn
Lablab purpureus	118.3c	167.0b	104.2c	14.8c	39.0b	8.8b	33.2c	214.4c	42.3b	42.3c
Leucaena leucocephala	338.0b	189.8a	137.3a	44.4a	44.6a	12.6a	52.6a	642.4a	55.0a	125.1a
Mucuna utilis	443.0a	105.0c	115.6b	22.0b	36.0c	8.8b	47.8b	239.1b	20.5c	61.5b

Table 3: The saponification, Iodine and Acid values of seeds of *L. purpureus, L. leucocephala* and *M. utilis*

	(mg/100 g)		
Seed types used	Saponification value	Iodine value	Acid value
Lablab purpureus	72.81±4.0c	13.85±1.5a	6.40±1.64a
Leucaena leucocephala	108.74±2.0a	4.90±0.6b	1.08±0.05c
Mucuna utilis	86.36±6.5b	14.96±1.62a	5.09±0.42ab

Each value is a mean of four replicates,
Figures followed by same alphabet along the columns are not significantly different at p = 0.05 using DMRT to separate the means

The seeds of *L. leucocephala* contained the highest saponification value followed by *M. utilis* and tailed by seeds of *L. purpureus*. The seeds of *L. leucocephala* contained the least amount of acid and iodine values, which are significantly less (p = 0.05) than those of *L. purpureus* and *M. utilis* (Table 3).

DISCUSSION

The presence of sugars in these seeds indicate that necessary materials needed to liberate energy during tissue respiration are readily available which guarantee readiness for energy supply in aid of seed germination. The presence of the various mineral nutrients such as Ca, Mg, K, N, Na, Zn, Cu, Fe and Mn are of biochemical importance to the physiology of the seeds. Nitrogen is a common constituent of protein synthesis, nucleic acid, RNA and DNA. Phosphorus is a constituent of co-enzyme NADH and NAD. Which are important energy producing units in biomembranes as in (ATP) Adenosine triphosphate. Calcium is important in cell-wall formation, and in the formation of cell membranes, lipid structures. Calcium is involved in normal mitosis thus, ensuring non-occurrence of abnormalities in seeds and plants [19]. Magnesium is an important constituent of chlorophyll molecule which ensures non-discolouration of young seedlings. Webster and Varnera [20], reported that potassium is essential as an activator for enzymes involved in the synthesis of certain peptide bonds. Iron functions in the synthesis of chloroplastic protein and may impair the machinery for chlorophyll synthesis [21, 22]. Manganese plays an important role in respiration and nitrogen metabolism, while copper acts as a component of phenolases, laccase and Ascorbic acid oxidase [19]. Zinc is involved in the biosynthesis of plant auxin-iodole-3-Acetic acid (IAA) and participates in the metabolism of plants as activator of growth [19]. Taking into consideration the various physiological contributions of these mineral nutrients to seedling development, it may be deduced that these seeds have enough materials to aid germination and growth if inhibitors are removed from the testa.

The oil contents obtained from *L. leucocephala* contain saturated fatty acid which would solidify as fats at a low temperature. The lipid content also have significantly high saponification value of 108.74 mg/100 g, which is higher than the recommended value of 100 mg/g by the British Pharmacopoeia [23] and Paranjpe [24]. The low iodine value is an indication that the fats are non-drying and would be good for cooking. The low acid value denotes its suitability for consumption. These observations are similar to that of Alabi *et al.* [25] and Ayelaagbe *et al.* [26].

From these studies it has been made clear that seeds of *Leucaena leucocephala* would be advantageous to man either for consumption and for small scale industrial set up in oil production.

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Response of Maize (*Zea mays*) and Okra *(Abelmoschus esculentus)* Intercrop Relayed with Cowpea (*Vigna unguiculata*) to Different Levels of Cow Dung Amended Phosphate Rock

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Abstract: Field trial was conducted at the Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria in 2002 and 2003 to assess the effect of Ogun rock phosphate (ORP) amended with cow dung (CD) manure on the growth and yields of maize and okra in intercrop relayed with cowpea on an Aquic Arenic Haplustalf. Significant treatment effects were observed in plant height and leaf area of maize and okra whereas stem girth was not significantly affected in either crop. The percentage leaf P concentration of maize, okra and cowpea were significantly ($p<0.05$) affected by treatment application. The percentage ranged from 0.18-0.48 and 0.24-0.45 in maize, 0.20-0.39 and 0.21-0.40% in okra and 0.16-0.40 and 0.18-0.42% in cowpea in 2002 and 2003, respectively. Increase in available P in amended ORP over sole ORP ranged from 44-71, 40-71 and 50-67% in the 2nd, 3rd and 4th sampling period. The ORP + 4 t haG[1] CD gave the highest P content of leaf in all the crops and in both years. The complementary use of Ogun rock phosphate with 3 t haG[1] cow dung manure produced the highest yields of maize (3.2 and 2.3 t haG[1]), okra (1.6 and 2.5 t haG[1]) and cowpea (1.8 and 1.9 t haG[1]) in 2002 and 2003, respectively.

Key words: Amended % cowpea % intercropping % Ogun rock phosphate % okra % maize

INTRODUCTION

In many developing countries, traditional agricultural systems are based on the growing of crops in mixture [1]. Multiple cropping is the intensification of crops in time and space by growing crops simultaneously on the same piece of land in a year [2]. According to Agboola [3], multiple cropping is the most dominant cropping system in Nigeria and it is the best cropping system for the soil of the humid tropics. He stated further that resources available to the farmers are well matched in maintaining low but often adequate and relatively steady production. In Nigeria, crop combinations vary a great deal from one ecological zone to another. One of the common combinations in Southwestern Nigeria is maize/okra [4].

The low nutrient status of most tropical soil necessitates the use of fertilizers for intensive cropping systems [5]. The importance of phosphorus (P) as yield limiting factor in many Nigerian soils is well established [6 - 9]. However the basic information required for designing of annual or seasonal maintenance of P fertilizer rates for an intercrop of most tropical soil is still inadequate. To date very little is known about the aggregate P requirement or maize/okra intercropping to the extent that fertilizer P recommendation have so far been single crop oriented. The crops, however, may respond differently to P, when double cropped due to each using the residual P applied to the previous crop.

The high cost of soluble phosphate fertilizer, such as single super phosphate, has generated considerable interest in the utilization of rock phosphate (RP) [10]. Concerns are often expressed on the effectiveness of RP for direct application. However, direct application of ground rock phosphate had been proved to be beneficial to crops on acid soils. Numerous studies have been conducted amending rock phosphates to increase their immediate P availability and also possibly enhance their rate of dissolution after application to soil. Composting of RPs with agricultural waste is known to increase solubility of rock phosphates [11-13]. The extent of P solubilization of a given RP varies with the kinds and the

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rate of decomposition of the organic material used [11]. For instance, Akande *et al*. [14] evaluated the comparative effect of urea and poultry manure (PM) on solubilization of rock phosphate and on the growth and yield of okra (*Abelmochus esculentus*, (L), *Moench*). Okra growth and yield were significantly enhanced by the addition of the treatments. The use of rock phosphate combined with poultry manure and to a lesser degree Urea, significantly improved the growth and yield of okra compared to when the materials were used individually. Application of RP plus urea and RP + PM was also found to increased soil available P by between 112 and 115%; and 144 and 153%, respectively for two years field trials.

The objective of the present study was to evaluate the effects of cow dung manure on release of P from RP and yields of maize and okra in intercrop relayed with cowpea.

MATERIALS AND METHODS

Field trial was carried out in the Research Farm of Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria in the early and late growing seasons of 2002 and 2003. The site of the experiment lies between Latitude 7° 31'N, Longitude 3° 54'E. Mean annual rainfall which is bimodally distributed is 1350 mm. The soil is an Aquic Arenic Haplustalf.

The site was ploughed and harrowed. Soil samples for chemical analysis were randomly collected from the top 15 cm depth prior to cropping in 2002. Soil analysis results were 6.0 pH (water) and 0.34, 0.32, 2.27, 0.55 cmol kg^{-1} for exchangeable Na, K, Ca and Mg, respectively. The total N and available P were 0.9% and 4.39 cmol kg^{-1}, respectively. The cow dung was collected from the livestock unit of the institute. It was sun-dried and grinded. The cow dung chemical analysis results were 5.8 pH and 9.8, 1.68, 0.89, 1.69, 0.26 1.34, 0.08% for organic matter, N, P, K, Na, Ca and Mg, respectively. Soil samples were taken from the plots for chemical analysis following the first harvest in 2002 and prior to and after the second cropping harvest in 2003.

The design of the experiment was a Randomized Complete Block with three replications. The blocks were 1 m apart and each block consisted of six plots, each measuring 5 m x 3 m with a space of 0.5 m between plots. The treatments consisted of control (no fertilizer), Ogun rock phosphate (100 kg P_2O_5 ha^{-1}) alone and Ogun RP (100 kg P_2O_5 ha^{-1}) combined with four levels (1, 2, 3, 4 t ha^{-1}) of cow dung. Basal application of urea (100 kg N ha^{-1}) and muriate of potash (60 kg K_2O ha^{-1}) were mixed with the treatments.

Maize, CV, DMR-ESR-Y and okra, CV. V35 was intercropped. Two seeds were planted per hill with a spacing of 75 cm x 30 cm inter row. 1:1 method of planting was adopted for both crops. Herbicides (paraquat and cypermethrin) were used to control weeds at the rates of 40 and 100 mL, respectively per 15 L of water. Treatments were applied a week after seedling emergence. Nuvacron at the rate of 50 mL per 20 L of water was applied on okra plants to control insect attack at 3 week after planting. Plant height, stem girth, leaf area and fresh fruit of okra yield were determined. Leaf samples of both crops were taken for chemical analysis. For maize, the index leaf taken was the one directly opposite and below the ear leaf, while that of okra was recently mature leaf before the onset of flower initiation. Okra fruits were harvested at 3 days interval for eight consecutive weeks and fresh weight determined. The summation of total yield was calculated. Maize ear was allowed to dry on the stalk before harvesting. The cobs were dehusked, shelled and weighed. Yield per hectare was computed at 12% moisture content. In the late season of 2002, cowpea was planted on the plot to assess the residual effect. In 2003 the experiment was repeated.

RESULTS AND DISCUSSION

Agronomic parameters: Maize and okra plant height were significantly influenced by either sole or complementary use of rock phosphate and cow dung manure in both years of cropping (Table 1). Mean maize height was 190 and 180 cm in 2002 and 2003, respectively. The complementary application of ORP plus cow dung produced taller plants than the sole use of ORP. The percentage increase in maize heights under complementary use of ORP and cow dung over sole ORP ranged from 10-24 and 11-22% in 2002 and 2003, respectively. All treatments except sole ORP (in 2002) significantly increased okra plant heights in both years. Stem girth of maize was not significantly influenced by treatment application in the two years. Okra stem girth was significantly increased when ORP was combined with 3 and 4 t ha^{-1} of cow dung in 2002 and 4 t ha^{-1} of cow dung in 2003. Leaf area of maize was positively affected by treatment application in both years. Amended ORP obtained significant higher leaf area of maize than un-amended ORP in each year of cropping. However, there was no significant difference among the rates of cow dung used. Whereas, there was significant difference in okra leaf area due to the treatments applied, there was no significant difference among the treatments except 2 and 3 t ha^{-1} CD in 2002 and 3 t ha^{-1} CD in 2003 which gave statistically higher leaf area than ORP.

Table 1: Effects of treatments on growth parameters of maize and okra

	2002						2003					
	Maize			Okra			Maize			Okra		
Treatments	Height (cm)	Stem girth (cm)	Leaf area	Height (cm)	Stem girth (cm)	Leaf area	Height (cm)	Stem girth (cm)	Leaf area	Height (cm)	Stem girth (cm)	Leaf area
Control	154.0c	4.8a	361.0d	36.0b	3.7b	109.0c	140.0c	4.5a	313.0d	38.0c	3.5bc	88.0c
ORP	174.0b	5.5a	388.0c	39.0ab	4.2ab	132.0b	166.0b	4.9a	412.0c	50.0b	4.6b	112.0b
ORP+CD1	203.0ab	5.5a	402.0b	44.0a	4.3ab	136.0ab	189.0b	5.1a	464.0b	59.0ab	5.1ab	127.0ab
ORP+CD2	216.0a	6.0a	570.0a	44.0a	4.5ab	158.0a	184.0b	5.4a	509.0ab	57.0ab	5.3ab	133.0ab
ORP+CD3	202.0ab	5.8a	537.0ab	45.0a	4.7a	161.0a	200.0a	5.8a	538.0a	64.0a	5.8ab	147.0a
ORP+CD4	192.0ab	5.4a	538.0an	43.0a	4.9a	144.0ab	203.0a	5.9a	554.0a	55.0ab	6.3a	126.0ab

Table 2: Phosphorus concentrations (%) in leaves of maize, okra and cowpea

	2002			2003		
Treatments	Maize	Okra	Cowpea	Maize	Okra	Cowpea
Control	0.18d	0.20c	0.16d	0.24c	0.21d	0.18d
ORP	0.30c	0.28b	0.24c	0.35b	0.29c	0.26c
ORP+CD1	0.40b	0.34ab	0.30b	0.40ab	0.34b	0.30ab
ORP+CD2	0.45ab	0.36a	0.34ab	0.43a	0.36ab	0.34ab
ORP+CD3	0.46ab	0.38a	0.35ab	0.44a	0.38a	0.40a
ORP+CD4	0.48a	0.39a	0.40a	0.45a	0.40a	0.42a

Table 3: Effect of treatments on soil available P (mg kg^{-1})

	Prior to cropping		
Treatments	2nd	3rd	4th
Control	3.39d	2.48d	1.98c
ORP	6.54c	8.76c	10.14b
ORP+CD1	9.42b	12.29b	15.23ab
ORP+CD2	9.89b	12.77b	15.44ab
ORP+CD3	10.48a	14.38a	16.48a
ORP+CD4	11.21a	14.98a	16.96a

Table 4: Effects of treatments on grain yield (t ha^{-1}) of maize, okra and cowpea

	2002			2003		
Treatments	Maize	Okra	Cowpea	Maize	Okra	Cowpea
Control	1.1d	0.4c	0.8c	0.3d	0.3d	0.6c
ORP	1.8c	0.7b	1.2b	0.9c	1.2c	1.3b
ORP+CD1	2.7b	1.1b	1.4ab	1.2b	1.6b	1.6ab
ORP+CD2	3.0ab	1.4ab	1.7a	1.5b	1.9ab	1.8a
ORP+CD3	3.2a	1.6a	1.8a	2.5a	2.3a	1.9a
ORP+CD4	2.7b	0.9b	1.6a	2.2a	1.6b	1.7ab

Means having the same letter(s) in a column are not significantly (p=0.05) different according to DMRT

Phosphorus content in plant leaves: The percentage P content in maize, okra and cowpea leaves sampled after treatment application are presented in Table 2. Significant increases in leaf P concentration were produced in all the crops by the treatment applied. In the case of maize, the percent leaf P concentrations ranged between 0.18 and 0.48 in 2002 and 0.24 and 0.45% in 2003. Amendment of ORP with CD significantly increased leaf P concentration. There was a slight increase in P content as the rate of cow dung increased though this was not significant except between ORP plus CD1 and ORP plus CD4 in 2004. ORP + CD_4 produced the highest P content in both years. The percentage P content in okra leaves ranged from 0.20-0.39 and 0.21-0.40 in 2002 and 2003, respectively. The trend was similar to that of maize plant. The percentage increase in leaf P content of okra treated ORP plus different rates of cow dung over sole ORP ranged from 21-39 and 17-38% in 2002 and 2003, respectively. The percentage P content in cowpea leaves ranged from 0.16-0.40 and 0.18-0.42% in 2002 and 2003, respectively. It was observed that as the rate of cow dung increased the P concentration also increased in both years for all the crops.

Soil available phosphorus: After the first cropping, soil P had significantly increased by all the treatments except the control that declined (Table 3). Cow dung amendment produced significant increase in soil available P above un-amended ORP. The percentage increase ranged from 44-71, 40-71 and 50-67% in prior to the 2nd, 3rd and 4th cropping, respectively. Also, the percentage increase in sole ORP over the control were 93, 338 and 513 in prior to 2nd, 3rd and 4th cropping, respectively.

The soil available P increased with increasing rate of CD. Soil available P with 3 and 4 tonnes CD was similar and statistically higher than 1 and 2 tonnes CD that were also similar. The same trend was sustained after the second cropping, prior to the third cropping. Prior to the fourth cropping, the results still showed significant increase in soil available P due to treatments applied. However, the effect of amending ORP with CD was no longer significant, showing that amendment sustaining the soil available P increase for three consecutive crops. The efficacy of cow dung in facilitating the release of P from applied rock phosphate resulted in significantly higher available P than using rock phosphate alone. This must have been responsible for the remarkable yield increase observed from the co-application of ORP and cowdung. Similar yield increases of maize and cowpea has been reported by

Akande *et al*. [15] through the combined use of rock phosphate composted with poultry manure. The increase in P availability observed through amendment of rock phosphate with organic materials was also explained by Khanna *et al*. [16], as resulting from the conversion of rock phosphate P to water soluble form and greater efficiency of the dissolved P in terms of availability to plant.

Yields of maize, okra and cowpea: Ogun rock phosphate significantly improved grain yield of maize resulting in as much as 63 and 200 % yield increases in 2002 and 2003, respectively (Table 4). Cow dung applied along with ORP resulted in further significant yield increases, the highest being obtained when CD was applied at 3 t ha^{-1} in the two years of cropping. This was significantly higher than ORP+CD1 and ORP+CD4 both of which produced statistically similar maize grain yield. The CD at optimum level of 3 t ha^{-1} increased yield by 77.7 and 160 % over and above sole ORP in 2002 and 2003, respectively.

Fresh fruit yield of okra was also markedly improved by application of ORP solely or in combination with CD. Only 3 t ha^{-1} CD gave significantly higher yield than sole ORP in the two years of cropping. Other rates of CD were not significantly different. Cowpea followed a somewhat similar trend.

Treatments involving CD gave significantly higher yields than sole ORP except CD1, in the first year and CD4 in the second. No significant difference, were observed among all the rates of CD used. Maize yield were higher in 2002 than in 2003, this could be due to dearth of rainfall observed after the trial establishment in 2002 whereas the reverse was the case for okra and cowpea.

The results of the present study showed that ORP amended with cow dung manure was superior to the control and sole application of ORP. This shows that the effectiveness of ORP on crop production was remarkably improved through the solubilizing effect of cow dung manure. Furthermore, the complementary use of rock phosphate with 3 t ha^{-1} cow dung manure gave the optimum yield in maize and okra intercropped relayed with cowpea, while above this rate there was declined in yield of the component crops.

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