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EFFECT OF BIOFERTILIZER ON QUALITY AND QUANTITY OF NITROGENOUS COMPONENTS, OIL CONTENT AND ITS QUALITY OF PEANUT (*ARACHIS HYPOGAEA* L.) UNDER CONDITIONS OF EL-SHEIKH ZUWAYID REGION

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ABSTRACT

This Investigation was conducted under new reclaimed area at El-Sheikh Zuwayid Research Station, North Sinai, Desert Research Center during two successive seasons of 2008 and 2009. The main objective was to study the effect of biofertilizer .i.e [50% Microbein (MB) + 50% Chemical fertilizer (RCF)] , [75% Microbein (MB) + 25% Chemical fertilizer (RCF)] and the recommended dose of chemical fertilizer (RCF) as a control on quality and quantity of nitrogenous components, oil content and its quality of peanut (Giza5, Giza6 and Asmailia1). **The results showed that:-**

- The highest values of weight of 100 seeds, pod yield, seed yield, oil yield and proteins yield were found under the treatment of Giza6 with (75% MB + 25% RCF), followed by treatment of Asmailia1 with (75% MB + 25% RCF).
- Giza 5 exceeded all varieties in total and soluble proteins. Biofertilizer treatments significantly enhanced proline and proteins fraction. Treatment of (75% MB + 25%RCF) produced the highest value of proline and proteins fraction.
- Indol-3-acetic acid (IAA), gibberellic acid (GA₃) and (GA₃+IAA) concentrations were increased by application of biofertilizer for all peanut varieties. Meanwhile, ABA content showed an opposite trend. Giza5 plants treated with (75%MB+25%RCF) recorded the maximum value of GA₃ and (GA₃+IAA) followed by Giza6 with (75% MB + 25%RCF). IAA in shoots of Giza6 recorded the highest value due to (75% MB + 25%RCF) treatment.

- The highest values of K and K/Na were obtained by the combination between (75% MB + 25% RCF) and Asmailia1 variety. While, the same treatment was recorded the lowest mean value of Na content in the same variety. Application of (75% MB + 25% RCF) gave the highest value of calcium in shoots of Giza5, followed by Giza6 with (75% MB + 25% RCF) then Giza6 with (50% MB + 50% RCF).
- The results of SDS-electrophoretic pattern of total proteins extracted from seeds revealed that molecular weight of protein sub units ranged between 15 to 200 kDa. The major variations are expressed as changes in band intensity, appearance or disappearance of some bands. Bands of molecular weight 114, 115, 120, 150 and 170 kDa were not presented in all samples of Giza5 and Asmailia1 varieties (in case of control and biofertilizer), but the same bands were not presented in the samples of Giza6 after treatment with biofertilizer.
- Application of (75% MB + 25% RCF) treatment gave the highest values of oil% in seeds of all varieties. Also, the maximum value of total proteins was produced by Giza6 with (50% MB + 50% RCF). Asmailia1 variety treated with (75% MB + 25% RCF) recorded the maximum value of soluble proteins followed by Giza6 with (50% MB + 50% RCF).
- Biofertilizer treatments affected negatively on acid value and saponification value for all peanut varieties. Giza6 recorded the lowest mean value for acid value after treatment with (75% MB + 25% RCF), and recorded the lowest value of saponification value when treated with (50% MB + 50% RCF).

Keywords: Peanut (*Arachis hypogaea* L.), Biofertilizer, Proteins, Hormones, Minerals, SDS-PAGE, Oil.

Abbreviations: MB, microbein; RCF, recommended dose of chemical fertilizer; IAA, indol-3-acetic acid; GA₃, gibberellic acid; ABA, abscisic acid

INTRODUCTION

The genus *Arachis*, a member of the family Leguminosae, is widely distributed in the tropics and moderate region. Peanut (*Arachis hypogaea* L.) is an important source of edible oil for millions of people living in the semi tropic region. The peanut plant is known to prefer hot climates with sandy soils. Consequently, nutrients disorders in these soils are the most important limiting factor to crop production.

Imbalance plant nutrition is also one of the major constrains for attaining maximum crop yield .Major problems are deficiencies of nitrogen and phosphorus; however, recent research has revealed that micronutrient problems are also hampering crop production. Most of the cultivated area in poor sandy soil using high rates of NPK chemical fertilizers aiming to maximize seed and straw yield. Biofertilizers are microbial preparations containing primary sufficient number of active strains of microorganisms having an explicit role in furnishing better rizosphere for plant growth. Nasef *et al* (2006) found that biofertilizer (Rhizobium strains) improved the growth and economic yield of groundnut, also increased the uptake of N, P, K, Mn, Zn and B by straw and seeds of peanut. In addition, increasing groundnut yield and its components by using the integrated of both bio-organic and chemical fertilizers were reported by El Kramany *et al* (2007).

Peanut is an important oil and food crop, and is grown primarily for human consumption either as whole seeds or processed to make peanut butter, oil and other products. Peanut seeds are a rich source of edible oils and contain about 42 to 52% oil, 25 to 32 % proteins and provide minerals such as phosphorus, calcium, magnesium, potassium and vitamins [Savage and Keenan (1994), Nath and Alam (2002) and Ahmad and Rahim (2007)]. Recently, the use of groundnut meal is becoming more recognized not only as a dietary supplement for children on protein poor cereals-based diets but also as effective treatment for children with protein related malnutrition. Seed oxidative stability is closely associated with oil composition; therefore, fatty acid composition in peanut seed is an important quality attribute, regardless of whether the seed is used as food or oil. The two predominant fatty acids in the peanut seed oils are oleic acid and linoleic acid which together comprise about 80 percent of peanut fatty acid composition. The oil is pale yellow and has the characteristic odor and flavor of peanuts (O'Brien 1998). Oil quality and its stability are therefore very important for the consumers (Jambunathan *et al* 1993). Also, the groundnut oil may have a higher shelf life, and serve as a useful substitute in nutrition and industrial applications (Anyasor *et al* 2009).

Therefore, the current research was designed to evaluate the effect of biofertilizer on quality and quantity of nitrogenous components, oil content and its quality of peanut.

MATERIALS AND METHODS

Two field experiments were carried out during 2008 and 2009 seasons in El-Sheikh Zuwayid Research Station, North Sinai, Desert Research Center, to perform this study. The experimental sites soil was sandy and irrigated through a drip irrigation system with water having 2636 ppm salts. Table (1) shows mechanical and chemical characteristics of the studied soil and irrigation water. The organic fertilizer was added to all experimental plots at a rate of 20 m³/fed. (as recommended dose) during soil preparation. Organic manure was mixed with the soil surface before cultivation. The recommended dose of chemical fertilizer (RCF) was used as control i.e. nitrogen fertilizer was added at a rate of 200kg/fed. ammonium sulfate (20.6%N) in two equal parts at 15 and 45 days after sowing, while phosphorus and potassium were added at a rate of 200 kg/fed. calcium superphosphate 15.5%P₂O₅ and 100 kg/fed. potassium (48% K₂O), respectively (El Kramany *et al* 2007).

The studied main factors were:-

1-Biofertilizer treatments were as follows:

- Control, the recommended dose of chemical fertilizer [RCF].
- 50% Biofertilizer (Microbein) + 50% recommended dose of chemical fertilizer [50% MB+50%RCF].
- 75% Biofertilizer (Microbein) + 25% recommended dose of chemical fertilizer [75% MB+25%RCF].

The Microbein is biofertilizer innovated and identified by Saber (1993), fecund by biofertilizers unit, General Organization of Agriculture Equalization fund (GOAEF) oversight Ministry of Agriculture, Egypt, contains *Azotobacter spp*; *Azospirillum spp*; *Pseudomonas spp*; *Bacillus megatherium*; *Rhizobium spp*.

2- Peanut varieties

Three peanut varieties (Giza5, Giza6 and Asmailia1) were obtained from the Field Crop Institute, Agricultural Research Center, Ministry of Agriculture, Egypt and sown in second week of May in

Table 1. Mechanical and chemical analysis of the experimental soil and chemical analysis of irrigation water at El-Sheikh Zuwayid, North Sinai.

a) Mechanical analysis of the experimental soil.

Characters	Values
Fine sand (%)	30.64
Coarse sand (%)	59.99
Clay (%)	2.39
Silt (%)	6.98
Texture class	Sandy

b) Chemical analysis of the experimental soil.

Characters	Values
pH	7.91
E.C. (mmhos/cm)	0.90
Soluble cations (meq/L)	
Ca ⁺⁺	3.96
Mg ⁺⁺	0.88
Na ⁺	4.71
K ⁺	0.22
Soluble anions (meq/L)	
CO ₃ ⁼	----
HCO ₃ ⁻	1.82
Cl ⁻	3.05
SO ₄ ⁼	4.90

c) Chemical analysis of irrigation water.

Characters	Values
pH	7.72
E.C. (mmhos/cm)	4.12
Soluble cations (meq/L)	
Ca ⁺⁺	6.02
Mg ⁺⁺	7.67
Na ⁺	27.58
K ⁺	0.19
Soluble anions (meq/L)	
CO ₃ ⁼	-----
HCO ₃ ⁻	3.28
Cl ⁻	27.03
SO ₄ ⁼	11.15

both seasons. Seeds of groundnut were coated just before sowing with (Microbein) biofertilizer using Arab gum as an adhesive agent.

The experimental design was split plot with 3 replicates in both seasons. The experiment included 9 treatments which were the combination of 3 biofertilizer and 3 peanut cultivars.

Sampling

Plant samples were taken randomly from each treatment after 55 days from sowing. Fresh samples were tested for proline and plant hormones. Then, dried till constant weight representing dry weight samples were grounded to fine powder and tested for proteins and minerals contents.

Harvesting

Plants were harvested after 150 days from sowing, the following components were recorded: weight of 100 seeds (g), seed yield (kg/fed.), oil yield (kg/fed.) and proteins yield (kg/fed.). Chemical analysis of seeds was conducted to determine oil %, proteins % and proteins patterns. Furthermore, physicochemical properties of peanut oil.

Chemical analysis

Determination of proteins

The total proteins were determined in dried sample using micro Kjeldahl method described in A.A.C.C. (1994). Soluble proteins content was determined according to Lowry *et al* (1951). The non-soluble proteins were calculated by subtracting the amount of soluble proteins from the total protein content.

Determination of free proline

Free proline concentration was measured calorimetrically in the extract of fresh materials according to Bates *et al* (1973).

Determination of oil content and physicochemical properties of peanut oil

Oil content in peanut seeds as well as refractive index, acid value and saponification value of peanut oil were estimated according to A.O.A.C. (1995).

Determination of minerals

Sodium, Calcium and Potassium minerals were determined in the acid digested samples by using flame photometer (Jenway PFP7) according to Brown and Lilleland (1964).

Determination of growth hormones

The method used for extraction of growth hormones [Abscisic acid (ABA), Indole-3-acetic acid (IAA) and Gibberellic acid (GA₃)] was that originally described by Shindy and Smith (1975). The growth hormones were estimated by HPLC.

SDS-PAGE of proteins in seeds

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of total proteins was performed according to the method of Laemmli (1970) as modified by Studier (1973).

Statistical analysis

The data from all experiments were subjected to the Statistical analysis of variance and calculated means were separated by Duncan's (1955) multiple range test at 0.05 level, using MSTAT computer statistical software according to Russel (1991).

RESULTS AND DISCUSSION

A. Yield and yield components

The yield and some important yield components of peanut, i.e. weight of 100 seeds, pod yield, seed yield, oil yield and proteins yield are recorded in Table (2). It is clear from the data that there were significant differences between treatments for all studied characters. Giza 6 exceeded the other varieties (Giza5 and Asmailia1) in yield and yield components. Results in the same table show the effect of biofertilizer, the highest yield and yield components were obtained by using 75% MB (Microbein) + 25% RCF (recommended dose of chemical fertilizer) as compared with the control.

Concerning the effect of interaction between varieties and biofertilizer on yield and yield components of peanut plants, the highest values of the studied parameters, i.e. weight of 100 seeds, pod yield, seed yield and oil yield were found under the treatment of Giza6 with (75% MB + 25% RCF), followed by treatment of Asmailia1 with (75% MB + 25% RCF) as compared with the control. In this regard, the highest value of proteins yield was obtained by the combination between (75% MB + 25%RCF) and Asmailia1 variety which was 307.97 Kg/fed.

The positive effect of biofertilizer on yield and yield components of peanut plants may be due to the important role in improving soil fertility, plant production and its quality, decreases

production costs and environmental pollution. Moreover, biofertilizer enhances the plant hormones levels (indol-3-acetic acid and gibberellic acid) in peanut plants (Table 4), also the role of such treatment in the osmotic pressure adjustment of plants under stress conditions.

The effect of biofertilizer on increase yield and yield components is well documented by El Kramany *et al* (2007) on peanut. In this regard, Nasef *et al* (2006) showed that seed inoculation with *Rhizobium* enhanced plant growth and improved peanut yield and its components. Also, Pal (1986), Negm *et al* (1998), Srivastava (1999) and Baktash *et al* (2003) reported that inoculation the peanut seeds with *Rhizobium* strains spp. significantly increased the yield and yield components of peanut plants. However, El Boraie *et al* (2009) indicated that inoculation with *Rhizobium*, *Azotobacter* and *Bacillus megaterium* (PDB) increased peanut growth yield.

Table 2. Effect of peanut varieties, biofertilizer and their interaction on yield and yield components of peanut plants.

Treatments		Yield and yield components				
		Weight of 100 seeds (g)	Pod yield (Kg/fed.)	Seed yield (Kg/fed.)	Oil yield (Kg/fed.)	Proteins yield (Kg/fed.)
Varieties (V)						
Giza 5		82.62 b	1193.91 c	835.89 c	398.83 c	212.04 b
Giza 6		90.13 a	1403.51 a	1019.52 a	514.96 a	260.23 a
Asmailia 1		86.27 ab	1314.98 b	962.12 b	474.05 b	241.70 a
Biofertilizer (B)						
Control (RCF)		80.29 c	1052.67 c	810.54 c	382.69 c	198.79 c
50% MB + 50%RCF		87.07 b	1252.95 b	901.55 b	447.84 b	230.14 b
75% MB + 25%RCF		91.66 a	1606.77 a	1105.43 a	557.31 a	285.03 a
Interaction VxB						
Giza5	Control (RCF)	75.50 d	998.53 e	740.48 f	346.69d	179.56 e
	50% MB+50%RCF	83.45 be	1158.70 de	826.79 de	394.95cd	208.68 e
	75% MB+25%RCF	88.90 ab	1424.50 b	940.40 bc	454.87bc	247.88 cd
Giza6	Control (RCF)	84.69 bc	1125.80 de	879.49 c-e	410.89cd	216.35d e
	50% MB+50%RCF	90.32 ab	1363.43 bc	967.54 b	497.70b	265.10 bc
	75% MB+25%RCF	95.39 a	1721.32 a	1211.54 a	636.30a	299.25 ab
Asmailia1	Control (RCF)	80.69 cd	1033.70 e	811.67 ef	390.49cd	200.48 e
	50% MB+50%RCF	87.43 bc	1236.73 cd	910.34 b-d	450.89bc	216.66 de
	75% MB+25%RCF	90.69 ab	1674.51 a	1164.35 a	580.77a	307.97 a
MB= Microbein, RCF= Recommended dose of chemical fertilizer Organic fertilizer was added to all experimental plots at recommended dose. Values followed by the same letter(s) in columns are not different at $p < 0.05$ by Duncan's multiple range test						

In another study, Zalate and Padmani (2009)a&b noticed that seed inoculation with biofertilizers (Rhizobium+PSM) significantly increased the pod, haulm and biological yields, shelling percentage, oil yield, yield attributing characters and yield of groundnut. Also, Kausale *et al* (2009) showed that nodule number, dry matter plant⁻¹, pod and haulm yield of groundnut crop were increased with application of 100% RDF (25:50 N and P kg ha⁻¹, 10 t FYM ha⁻¹ and rhizobium or PSB seed inoculation). On the same line, Sethi and Adhikary (2009) showed that yield of *Arachis hypogea* and *Vigna radiata* due to *Rhizobium* inoculation was higher by 22% and 29% respectively over control. Also, Shinde *et al* (2008) found that the combination of *Rhizobium* and all PGPRs (plant growth promoting rhizobacteria) resulted in the greatest number of pods per plant (16.5) and pod yield per plant (21.2 g). In the same trend, Parkhe *et al* (2008) showed that the combined inoculation of Rhizobium + *P. fluorescens* was optimum for the improvement of seed yield parameters of groundnut. In another study, Khan *et al* (2009) showed that the application of NP level 27:69 kg/ha might be a promising approach to enhance groundnut yield under rainfed condition.

B. Chemical analysis

1. Proline in leaves and proteins content in shoots

Data listed in Table (3) showed that, Giza 6 had a higher proline content than other peanut varieties tested. While, Giza 5 exceeded all varieties in total and soluble proteins. On the other hand, the differences between peanut varieties in non-soluble proteins are not significant. As shown in the same table, it is obvious that biofertilizer treatments significantly enhanced proline and proteins fraction as compared with the control. Treatment of (75% MB + 25%RCF) produced the highest value of proline and proteins fraction.

The interaction effect between peanut varieties and biofertilizer can be deduced from tabulated data in Table (3). The results showed that, the highest value of proline content was produced from Giza6 after treatment with (75%MB+25%RCF) followed by Asmailia1 with (75% MB+25%RCF). Also, application of (75%MB+25%RCF) gave the highest values of total proteins and soluble proteins in shoots of Giza5 followed by Giza6 with (75%MB+25%RCF) then Giza5 with (50% MB+50%RCF) as compared with the control. However, application of (75% MB+25%RCF) induced increase in non-soluble

proteins in shoots of Giza6, and recorded the highest value (87.20 mg/g) followed by Asmailia1 with (50% MB+50%RCF).

The increase in proline content might act as an osmoregulator compound and its accumulation considered as an adaptive response to stress conditions. Also, free proline has been proposed as a principle osmotic gradient controller which is not directly involved in osmotic adjustment but plays an indirect role in osmoregulation in plant species by increasing hydration of the protoplasm (Kumar 1984). Also, Samuel *et al* (1985) found that proline content was increased in all plant samples which may be due to increased nitrogen metabolism and nucleic acid (DNA and RNA) in plant cell. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing subcellular structure (e.g. membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions. In this regard, Ramanjulu and Bartels (2002) showed that large numbers of compounds are synthesized, which play a key role in maintaining the osmotic equilibrium and in protection of membranes as well as macromolecules. These compounds include proline and sugars.

The positive effect of biofertilizer on proline content was mentioned by Abd El-Gawad *et al* (2009) on canola. However, Aly (2002) noticed that using Biomagic (7.5 g/L) combined with application of 90 kg N/fed and seed inoculation with (*Rhizobium leguminosarum* + *Bacillus megatherium*) resulted in the highest value of amino acids content in pea plant leaves. In addition, Ismail (2002) found that the highest value of amino acids content of pea leaves were obtained by using foliar spraying with Biomagic. However, Sheteawi and Tawfik (2007) showed that biofertilized plants (Biogin and Nitrobin) recorded higher values of proline, total amino acids and nitrogen content than non fertilized plants of mung bean under water regimes. In addition, Ozturk and Demir (2002), Hsu *et al* (2003) and Kishore *et al* (2005) concluded that proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stress.

In this concern, Abdalla (2002) stated that the content of proteins in faba bean increased with biofertilizer treatments and with increasing rates of P from 100-200kg superphosphate/fed. El-Sharkawy *et al* (2003) observed that the highest values of tuber protein and nitrogen contents were shown by the treatment of

Nitroben with 25 and 50% of the recommended NPK (40 N + 22.5 P₂O₅ + 96 K₂O kg/fed) of Jerusalem artichoke. In another study, El-Habbasha *et al* (2007) indicated that protein content in seed of faba bean was increased by the addition of 45kg P₂O₅/feddan to seeds inoculated by *Rhizobium* compared the other treatments. However, Neveen and Amany (2008) concluded that fungal infection and *Rhizobial* inoculation either alone or in combination with 50% or 75% of the recommended doses of N and/or P fertilizers increased proteins of faba bean.

Table 3. Effect of peanut varieties, biofertilizer and their interaction on proline and proteins fraction in shoots of peanut plants at 55 days after sowing.

Treatments		Proline content (μ mole /g fresh wt.)	Proteins fraction (mg/g dry wt.)		
			Total proteins	Soluble proteins	Non-soluble proteins
Varieties (V)					
	Giza 5	2.87 b	130.42 a	53.74 a	76.67 a
	Giza 6	3.21 a	119.75 b	44.82 ab	74.93 a
	Asmailia 1	2.94 b	112.51 b	42.73 b	69.78 a
Biofertilizer (B)					
	Control (RCF)	2.44 c	103.23 c	39.01 c	64.22 b
	50% MB + 50%RCF	3.03 b	122.28 b	44.41 b	77.87 a
	75% MB + 25%RCF	3.55 a	137.17 a	57.87 a	79.30 a
Interaction VxB					
Giza5	Control (RCF)	2.64 de	110.48 de	40.93 c	69.55 c
	50% MB+50%RCF	2.82 cd	130.37 bc	50.28 b	80.09 ab
	75% MB+25%RCF	3.17 bc	150.41 a	70.03 a	80.38 ab
Giza6	Control (RCF)	2.28 e	103.44 de	38.93 c	64.51 cd
	50% MB+50%RCF	3.38 b	115.38 cd	42.29 c	73.09 bc
	75% MB+25%RCF	3.98 a	140.44 ab	53.24 b	87.20 a
Asmailia1	Control (RCF)	2.40 e	95.78 e	37.17 c	58.61 d
	50% MB+50%RCF	2.90 cd	121.10 cd	40.67 c	80.43 ab
	75% MB+25%RCF	3.52 b	120.67 cd	50.35 b	70.32 bc
MB= Microbein, RCF= Recommended dose of chemical fertilizer Organic fertilizer was added to all experimental plots at recommended dose. Values followed by the same letter(s) in columns are not different at $p < 0.05$ by Duncan's multiple range test					

2. Plant hormones content in shoots

In Table (4) the concentrations of indol-3-acetic acid (IAA), gibberellic acid (GA₃) and abscisic acid (ABA) in shoots of peanut varieties after treatment with biofertilizer are given. It is evident that, IAA, GA₃ and (GA₃+IAA) concentrations were increased by application of biofertilizer for all peanut varieties. Meanwhile, ABA content showed an opposite trend under the same conditions. In this concern, Giza5 plants treated with (75%MB+25%RCF) recorded the maximum values of GA₃ and (GA₃+IAA) followed by Giza6 with (75% MB + 25%RCF) as compared with the control. In addition, IAA in shoots of Giza6 recorded the highest value due to (75% MB + 25%RCF) treatment, followed by Asmailia1 with (75% MB + 25%RCF) treatment.

It is obvious from the results that, biofertilizer treatments affected negatively on growth inhibitor (ABA) in shoots of all peanut varieties as compared with the control. In this connection, the minimum value of ABA was produced by Asmailia1 plants which treated with (75% MB + 25%RCF) followed by Giza6 with (75% MB + 25%RCF). Data in the same table clearly demonstrate that, the ratios between gibberellic acid to indol-3-acetic acid (GA₃/IAA) ranged from 10.37 for Giza6 with (50% MB + 50%RCF) to 14.94 for Giza5 with (50% MB + 50%RCF). The later value simply means that the GA₃ is more than 10 folds that of the IAA ones. In this regard, GA₃/IAA ratio was decreased after treatment with biofertilizer in shoots of Giza5 and Giza6. While, Asmailia1 variety showed an opposite trend under the same conditions. In this respect, YoungKeun et al (2003) showed that the lower parts of the branch recorded higher concentrations of endogenous hormones (growth promoters) after flowering compared to the upper parts of the branch in peanut. However, Asha and Rao (2001) showed that waterlogging generally decreased the ABA content in seeds of peanut and leachates. Also, they found that, in both seeds and leachates, four indole derivatives identical to IAA, indole-3-acetaldehyde, indole-3-aldehyde were identified chromatographically. Also, TsaiChi et al (2001) showed that the decline of IAA content in peanut was accompanied by an increase in the activity of cationic peroxidase (POD) isozyme, which

was correlated with an increase in cationic POD transcripts, and may also remove excess hydrogen peroxide.

Table 4. Effect of biofertilizer on Plant hormones content in shoots of peanut varieties at 55 days after sowing.

Treatments		Plant hormones				
		GA ₃ mg/100g fresh wt.	IAA mg/100g fresh wt.	ABA µg/100g fresh wt.	GA ₃ +IAA mg/100g fresh wt.	GA ₃ /IAA
Giza 5	Control (RCF)	5.25	0.377	47.36	5.62	13.92
	50% MB + 50%RCF	6.83	0.457	25.02	7.28	14.94
	75% MB + 25%RCF	8.20	0.646	15.30	8.84	12.69
Giza 6	Control (R CF)	4.20	0.388	56.12	4.58	10.82
	50% MB + 50%RCF	5.74	0.553	33.90	6.29	10.37
	75% MB + 25%RCF	7.83	0.733	10.23	8.56	10.68
Asmailia1	Control (RCF)	4.52	0.422	14.10	4.94	10.71
	50% MB + 50%RCF	6.50	0.527	12.58	7.02	12.33
	75% MB + 25%RCF	7.50	0.690	7.12	8.19	10.86
GA ₃ = Gibberellic acid, IAA= Indol-3-acetic acid and ABA= Abscisic acid MB= Microbein, RCF= Recommended dose of chemical fertilizer Organic fertilizer was added to all experimental plots at recommended dose. Values followed by the same letter(s) in columns are not different at p< 0.05 by Duncan's multiple range test						

3. Minerals content in shoots

It is evident from the data presented in Table (5) that, Asmailia1 recorded the highest value for K/Na ratio and recorded the minimum values for Na and Ca as compared with the other peanut varieties.

It is quite clear from results that biofertilizer treatments significantly enhanced K, K/Na and Ca in shoots of peanut plants as compared with the control. On the other hand, Na content showed an opposite trend with biofertilizer treatments. However, (75% MB + 25%RCF) treatment produced the highest mean values for K, K/Na and Ca which reached 5.57 %, 3.20 % and 0.74 %, respectively. Meanwhile, the same treatment detected the lowest mean value for Na content which reached 1.77%.

Table (5) shows the effect of interaction between peanut varieties and biofertilizer on minerals content in shoots of peanut

plants, the highest values of K and K/Na were obtained by the combination between (75% MB + 25%RCF) and Asmailia1 variety which were 5.89 % and 3.97 %, respectively. On the other hand, Asmailia1 with the same biofertilizer treatment recorded the lowest mean value of Na content. The data also revealed that, application of (75%MB+25%RCF) gave the highest value of calcium in shoots of Giza5 which reached 0.86 %, followed by Giza6 with (75%MB+25%RCF) then Giza6 with (50% MB+50%RCF) as compared with the control.

Table 5. Effect of peanut varieties, biofertilizer and their interaction on Minerals content in shoots of peanut plants (*Hypogea archais*.L) at 55 days after sowing.

Treatments		Minerals content (g% dry wt.)			
		Na	K	K/Na ratio	Ca
Varieties (V)					
	Giza 5	2.23 b	4.53 a	2.11 b	0.69 a
	Giza 6	2.53 a	4.62 a	1.93 b	0.66 a
	Asmailia 1	1.93 c	4.94 a	2.70 a	0.43 b
Biofertilizer (B)					
	Control (RCF)	2.60 a	3.86 c	1.52 c	0.45 c
	50% MB + 50%RCF	2.32 b	4.66 b	2.01 b	0.58 b
	75% MB + 25%RCF	1.77 c	5.57 a	3.20 a	0.74 a
Interaction VxB					
Giza 5	Control (RCF)	2.69 a	3.69 d	1.37 d	0.55 ef
	50% MB + 50%RCF	2.12 b	4.56 c	2.15 c	0.67 cd
	75% MB + 25%RCF	1.89 b	5.34 ab	2.82 b	0.86 a
Giza 6	Control (RCF)	2.94 a	3.41 d	1.15 d	0.51 f
	50% MB + 50%RCF	2.71 a	4.97 bc	1.83 c	0.69 c
	75% MB + 25%RCF	1.95 b	5.49 ab	2.81 b	0.78 b
Asmailia1	Control (RCF)	2.17 b	4.48 c	2.06 c	0.31 h
	50% MB + 50%RCF	2.15 b	4.47 c	2.07 c	0.40 g
	75% MB + 25%RCF	1.48 c	5.89 a	3.97 a	0.60 de
MB= Microbein, RCF= Recommended dose of chemical fertilizer Organic fertilizer was added to all experimental plots at recommended dose. Values followed by the same letter(s) in columns are not different at $p < 0.05$ by Duncan's multiple range test					

Plants generally require mineral nutrients in all metabolic processes, e.g. K ions which is a part from its role as an osmotic component, K is essential for the formation of starch, protein synthesis, photosynthate partitioning, normal stomatal function and above all as an activator to a number of monovalent cation requiring enzyme (Marschner 1995 and Mahajan and Tuteja 2005). Also, calcium is important for cell wall, membrane, pectinates, and regulation of cell metabolism.

In this regard, Sheteawi and Tawfik (2007) showed that potassium content was higher by biofertilizer treatment (Biogin and Nitrobin) than non fertilized mung bean plants. In this concern, Vijaya and Srivasuki (2001) stated that dual inoculation with *Glomus macrocarpus* and *Bacillus megatherium* was superior to individual inoculation either with *G.macrocarpus* or *B. megatherium* in enhancing the nutrient uptake (Ca, Na and K) of micropropagated teak plants. Also, Abdel-Hameed (2002) on olive trees revealed that the interaction between 100% nitrogen fertilizer, biofertilizer (namely *Azotobacter spp.* + *Azospirillum spp.* and *Bacillus spp.*) and biostimulant (Biomagic) gave the highest significant leaf content of K. However, Mansour *et al* (2002) showed that biofertilizer (Nitrobin and Phosphorein) on globe artichoke plants increased the percentage of K element. While, Akath-Sing and Sing (2004) on olive tree found that application of (560g N + *Azotobacter chroococcum* + 500g of P and 500g of K/tree) or (375g of P + vesicular arbuscular mycorrhiza + 750g N + 500g K/tree) maintained the optimum level of K, Ca and Mg. Meanwhile, El-Etr *et al* (2004) found that compost plus the soil inoculated with *Azotobacter chroococcum*, *Bacillus megatherium* or *Bacillus circulans* increased in K uptake by pea and wheat plants. In addition, Salama (2005) found that using microbial mixture (*Azotobacter spp.* + *Azospirillum spp.* + *Bacillus megatherium*) and spraying Biomagic, especially in the presence of high level of organic manure (40m³/fed.) and high level of nitrogen (200 kg/N fed.), caused a high accumulation K in leaves of globe artichoke. Zaki and Salama (2006) reported that using the combined treatment of EM (bio-fertilizer) to soil and as foliar application on the cucumber plant increased K leaf content.

4. SDS polyacrylamide gel electrophoresis pattern for proteins analysis

The SDS electrophoretic patterns of total proteins extracted from seeds are summarized in Table (6) and illustrated in Fig. (1). From table and fig., it is quite clear that the molecular weight of proteins sub units ranged between 15 to 200 kDa. Also, Fig. (1) shows the more intensive band was presented at molecular mass 70 kDa.

Different treatments induced considerable variations in protein banding patterns. The major variations are expressed as changes in band intensity, appearance or disappearance of some bands. Bands of molecular weight 114, 115, 120, 150 and 170 kDa were not presented in all samples of Giza5 and Asmailia1 varieties (in case of control and biofertilizer), but the same bands was not presented in the samples of Giza6 after treatment with biofertilizer as compared with the control.

Also, bands of molecular weight 58, 80, 85 and 100 kDa were not presented in all sample of Asmailia1. In this regard, protein band having molecular weight 80 and 85 kDa for Giza6 were not presented in case of control and accumulated after treatment with biofertilizer treatment (50% MB + 50%RCF). Meanwhile, the same bands for Giza5 showed an opposite trend under the same conditions. Another protein bands with molecular weights 38, 48, 50 and 55 kDa were disappeared after treatment Giza6 variety with (75% MB + 25%RCF) as compared with the control. Also, one protein band having molecular weight of 38 kDa for Giza5 was disappeared in case of (50% MB + 50%RCF). On the other hand, bands of molecular weight 33 kDa for Giza6 and 30 kDa for Asmailia1 were accumulated in all biofertilizer treatments as compared with the control. In this regard, Sheteawi and Tawfik (2007) studied SDS-PAGE proteins banding pattern of mung bean seeds under different biofertilizer. They found that low level of protein polymorphism (9.6%). Thirty one bands were scored in the seed protein profile, twenty eight bands of them were monomorphic, while the remaining three bands were considered as polymorphic ones and were recorded at molecular weights 40.45, 18.40 and 15.27 kDa. In this regard, the use of sodium dodocyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique to separate the proteins banding patterns in peanut was reported by many authors such as Javaid et al (2004), Jain and Padmaja (2004), Anjana et al (2005), Kumari et al (2006), Choudhary et al (2007),

Davis et al (2007) , Nautiyal and Kulkarni (2009), Quist et al (2009) and Sathe et al (2009).

Table 6. SDS-PAGE patterns of proteins extracted from seeds of peanut varieties after treatment with biofertilizer.

Band number	Molecular weight (kDa)	Treatments								
		Giza 5			Giza 6			Asmailia 1		
		Control (RCF)	50% MB + 50% RCF	75% MB + 25% RCF	Control (RCF)	50% MB + 50% RCF	75% MB + 25% RCF	Control (RCF)	50% MB + 50% RCF	75% MB + 25% RCF
1	200	+	+	+	+	+	+	+	+	+
2	170	-	-	-	+	+	+	-	-	-
3	150	-	-	-	+	-	-	-	-	-
4	120	-	-	-	+	-	-	-	-	-
5	115	-	-	-	+	-	-	-	-	-
6	114	-	-	-	+	-	-	-	-	-
7	100	+	+	+	+	+	-	-	-	-
8	85	+	-	+	-	+	-	-	-	-
9	80	+	-	+	-	+	-	-	-	-
10	70	-	-	-	+	+	+	+	+	+
11	60	+	+	+	+	+	+	+	+	+
12	58	+	+	+	+	+	+	-	-	-
13	55	+	+	+	+	+	-	+	+	+
14	50	+	+	+	+	+	-	+	+	+
15	48	+	+	+	+	+	-	+	+	+
16	45	+	+	+	+	+	+	+	+	+
17	40	+	+	+	+	+	+	+	+	+
18	38	+	-	+	+	+	-	+	+	+
19	36	+	+	+	+	+	+	+	+	+
20	33	+	+	+	-	+	+	+	+	+
21	30	+	+	+	+	+	+	-	+	+
22	27	+	+	+	+	+	+	+	+	+
23	25	+	+	+	+	+	+	+	+	+
24	20	+	+	+	+	+	+	+	+	+
25	15	+	+	+	+	+	+	+	+	+
Total		20	17	20	22	20	13	15	16	16
MB= Microbein, RCF= Recommended dose of chemical fertilizer Organic fertilizer was added to all experimental plots at recommended dose.										

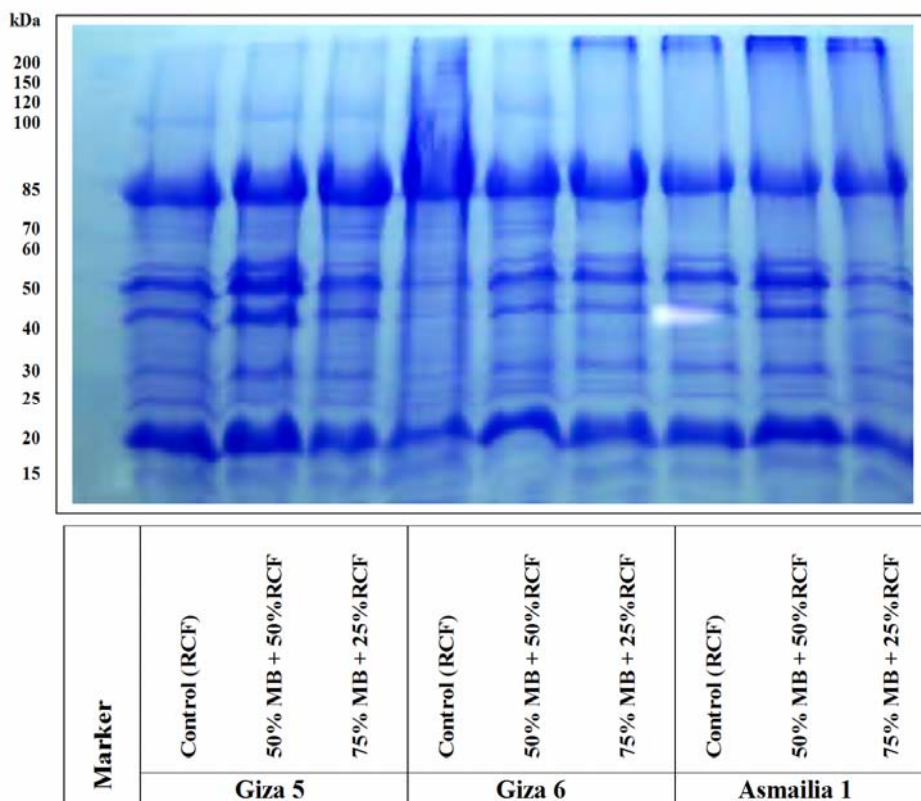


Fig (1): SDS-PAGE profiles of proteins extracted from seeds of peanut varieties after treatment with biofertilizer.

5. Oil content, physical & chemical properties of oil and proteins content in seeds

The differences among varieties performances were presented in Table (7). It was obvious that Giza6 recorded the highest mean values for oil content and soluble proteins which reached 50.23% and 10.24%, respectively. While, Giza6 and Asmailia1 recorded the minimum value of acid value and saponification value. Data showed that biofertilizer affected positively on oil, total proteins and soluble proteins, and negatively on acid value and saponification value. The highest values of oil, total proteins and soluble proteins were obtained by (75% MB + 25% RCF) treatment which reached 50.25, 25.84 and 10.73%, respectively. In this regard, Sheteawi and Tawfik (2007) showed that total crude proteins in seeds were higher by biofertilizer

treatment (Biogin and Nitrobin) than non fertilized mung bean plants. Also, Zalate and Padmani (2009)^{a&b} revealed that seed inoculation with biofertilizers significantly increased proteins content of *kharif* groundnut (*Arachis hypogaeae* L.). In another study, Reddy *et al* (2003) showed that groundnut is an important legume cash crop for the tropical farmers and its seeds contain high amounts of edible oil (43-55%) and protein (25-28%).

The effect of interaction between peanut varieties and biofertilizer can be deduced from tabulated data in the same table. Application of (75%MB+25%RCF) treatment gave the highest value of oil% in seeds of Giza6 followed by the same variety with (50%MB+50%RCF) as compared with the control. Also, the maximum value of total proteins was produced by Giza6 with (50% MB + 50%RCF) followed by Asmailia1 with (75% MB + 25%RCF) which reached 27.40 and 26.45%, respectively. In this direction, Asmailia1 plants treated with (75%MB+25%RCF) recorded the maximum value of soluble proteins followed by Giza6 with (50% MB + 50%RCF). Concerning the effect of interaction between peanut varieties and biofertilizer on chemical properties of peanut oil. It is quite clear from results that biofertilizer treatments affected negatively on acid value and saponification value for all peanut varieties. In this regard, Giza6 variety recorded the lowest mean value for acid value after treatment with (75%MB+25%RCF) which reached 1.81. Also, the same variety recorded the lowest value of saponification value when plants treated with (50% MB + 50%RCF).

Similar results were obtained by Abd El-Gawad *et al* (2009) they reported that the positive effect of biofertilizer treatment on oil quality is an expected result for its effect on improving physical and chemical properties of canola oil. Also, Yasari and Patwardhan (2007) noticed that the application of *Azotobacter* and *Azospirillum* helped increase the oil content of canola seeds. In reverse, El Bably and Awad (2007) found that increasing nitrogen fertilization significantly decreased the oil content in canola seeds. In addition, Farahbakhsh *et al* (2006) on rape (*Brassica napus* L.) and Kandil *et al* (2007) on groundnut, showed that nitrogen fertilizer affected the oil content negatively and decreased it.

Table 7. Effect of peanut varieties, biofertilizer and their interaction on oil content, physical & chemical properties of oil and proteins content in seeds of peanut plants.

Treatments	Oil %	Physical & chemical properties of oil			Protein content		
		Refractive index	Acid value	Saponification value	Total proteins %	Soluble proteins %	
<u>Varieties (V)</u>							
Giza 5	47.65 b	1.4575 a	2.17 a	192.1 a	25.28 a	8.83 b	
Giza 6	50.23 a	1.4582 a	1.99 b	188.6 b	25.57 a	10.24 a	
Asmailia 1	49.17 ab	1.4584 a	1.98 b	190.8 ab	24.98 a	10.09 ab	
<u>Biofertilizer (B)</u>							
Control (RCF)	47.22 b	1.4566 a	2.28 a	191.9 a	24.52 b	8.61 b	
50% MB + 50%RCF	49.58 a	1.4584 a	1.96 b	190.2 ab	25.48 ab	9.82 a	
75% MB + 25%RCF	50.26 a	1.4591 a	1.90 b	189.4 b	25.84 a	10.73 a	
<u>Interaction VxB</u>							
Giza 5	Control (RCF)	46.82 c	1.4565 a	2.41 a	192.4 ab	24.25 c	7.60 c
	50% MB + 50%RCF	47.77 c	1.4570 a	2.14 bc	193.3 a	25.24 bc	8.52 bc
	75% MB + 25%RCF	48.37 bc	1.4591 a	1.98 c-e	190.5 a-c	26.36 ab	10.39 ab
Giza 6	Control (RCF)	46.72 c	1.4565 a	2.33 ab	190.7 a-c	24.60 bc	9.01 bc
	50% MB + 50%RCF	51.44 ab	1.4591 a	1.84 de	186.5 c	27.40 a	11.51 a
	75% MB + 25%RCF	52.52 a	1.4591 a	1.81 e	188.5 bc	24.70 bc	10.21 ab
Asmailia 1	Control (RCF)	48.11 c	1.4570 a	2.10 b-d	192.6 ab	24.7 bc	9.22 bc
	50% MB + 50%RCF	49.53 a-c	1.4591 a	1.91 c-e	190.7 a-c	23.80 c	9.44b c
	75% MB + 25%RCF	49.88 a-c	1.4591 a	1.93 c-e	189.1 a-c	26.45 ab	11.60 a
MB= Microbein, RCF= Recommended dose of chemical fertilizer Organic fertilizer was added to all experimental plots at recommended dose. Values followed by the same letter(s) in columns are not different at $p < 0.05$ by Duncan's multiple range test							

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تأثير التسميد الحيوى على نوعية وكمية المركبات النتروجينية ومحتوى الزيت وجودته فى الفول السودانى تحت ظروف منطقة الشيخ زويد

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أقيمت تجربتان حقليتان خلال موسى 2008 ، 2009 بمحطة بحوث الشيخ زويد شمال سيناء التابعة لمركز بحوث الصحراء ، لدراسة تأثير التسميد الحيوى (50% ميكروبيين + 50% تسميد معدنى) ، (75% ميكروبيين + 25% تسميد معدنى) بالإضافة الى استعمال التسميد المعدنى الموصى به للمقارنة، على المركبات النتروجينية النوعية والكمية ومحتوى الزيت وجودته لثلاث أصناف من الفول السودانى (جيزة 5 ، جيزة 6 ، اسماعيليه 1) . وفيما يلى أهم النتائج:-

- أعطى صنف جيزة6 أعلى القيم لمكونات المحصول مثل وزن 100 بذرة، ومحصول القرون والبذور ، ومحصول الزيت والبروتين وذلك عند المعاملة بـ (75% ميكروبيين + 25% تسميد معدنى) ويلية صنف إسماعيلية1.
- أظهرت النتائج أن صنف جيزة5 تفوق على باقى الاصناف من حيث محتوى نباتات الفول السودانى من البروتين الكلى والذائب. وقد أدت معاملات التسميد الحيوى الى تحسن معنوى فى محتوى النباتات من البرولين الحر والبروتين، وقد أعطت معاملة (75% ميكروبيين + 25% تسميد معدنى) اعلى القيم فى محتوى النباتات من البرولين الحر والبروتين.
- أظهرت النتائج حدوث زيادة فى تركيز أندول أستيك أسيد (IAA)، والجبريلليك أسيد (GA3)، ونسبة (IAA+ GA3) وذلك عند المعاملة بالمخصب الحيوى الميكروبيين، بينما حدث نقص فى تركيز حامض الابسيسيك (ABA). وقد أعطى صنف جيزة5 أعلى القيم من (GA3) ، (IAA+ GA3) وذلك عند المعاملة بـ (75% ميكروبيين + 25% تسميد معدنى) ، ويلية صنف جيزة6 . كما سجل صنف جيزة6 أعلى القيم فى محتواه من أندول أستيك أسيد (IAA) وذلك عند معاملته بـ (75% ميكروبيين + 25% تسميد معدنى).
- وجد أن أعلى قيمة من عنصر البوتاسيوم ونسبة K/Na كانت واضحة وذلك عند معاملة صنف اسماعيلية1 بـ (75% ميكروبيين + 25% تسميد معدنى) وقد ظهرت أقل قيمة

لعنصر الصوديوم مع نفس المعاملة السابقة. وقد أظهرت النتائج أن إضافة المخصب الحيوي بالتركيز السابق يعطى قيم مرتفعة لعنصر الكالسيوم لاصناف جيزة 5، جيزة 6 ، اسماعيلية 1 بالترتيب.

■ دلت نتائج فصل البروتينات بالتحليل بواسطة SDS-PAGE على وجود حوالي 22 Bands ترواح وزنها الجزيئي من 15 الى 200 كيلودالتون. وكانت التغيرات الواضحة فى غياب أو ظهور بعض المناطق وكذلك التغير فى كثافتها. حيث وجد أن المناطق ذات الوزن الجزيئي 114، 115، 120، 150 كيلودالتون قد اختفت فى كل معاملات جيزة 5 ، اسماعيلية 1 (فى حالة الكونتروال والتسميد الحيوي)، ولكن فى حالة صنف جيزة 6 اختفت بعد المعاملة بالمخصب الحيوي (الميكروبي).

■ وجد أن معاملة الفول السودانى بـ (75 % ميكروبيين +25 % تسميد معدنى) يعطى أعلى قيمه لمحتوى البذور من الزيت. كما سجل صنف جيزة 6 أعلى القيم فى محتوى البذور من البروتين الكلى وذلك عن المعاملة بـ (ميكروبيين 50 % + 50 % تسميد معدنى). كما أعطى صنف إسماعيلية 1 أعلى القيم فى محتوى البذور من البروتين الذائب وذلك عند المعاملة بـ (75 % ميكروبيين +25 % تسميد معدنى) ، ويليه صنف جيزة 6 عند المعاملة بـ (50 % ميكروبيين +50 % تسميد معدنى).

■ أظهرت النتائج أن الخواص الكيميائية لزيت الفول السودانى مثل رقم الحامض ورقم التصبن قد تأثر سلبيا بمعاملات التسميد الحيوي. حيث وجد أن صنف جيزة 6 سجل أقل القيم لرقم الحامض وذلك عند معاملته بـ (ميكروبيين 75 % + 25 % تسميد معدنى)، كما سجل أقل القيم لرقم التصبن وذلك عن المعاملة بـ (ميكروبيين 50 % + 50 % تسميد معدنى) ويعطى هذا مؤشر جيد على جودة الزيت.

■ وتوصى هذه الدراسة بإمكانية زراعة الفول السودانى (صنف جيزة 6) ، صنف إسماعيلية 1 ، جيزة 5 بالترتيب) فى الاراضى الجديدة مثل منطقة شمال سيناء ذات التربة الرملية الخفيفة، وتوفير أكثر من نصف كمية التسميد المعدنى غالى الثمن وذلك باستعمال التسميد الحيوي (75 % ميكروبيين +25 % تسميد معدنى) الامن بيئيا ولخفض التكاليف الاقتصادية ونسبة التلوث، وارتباط ذلك بالمكونات البيوكيميائية المتعلقة بجودة محصول الحبوب والزيت.