GROWTH, NODULATION, YIELD AND MINERAL TISSUE CONTENT OF PEANUT IN RESPONSE TO FOLIAR AND COATING APPLICATION OF HUMIC ACID AND PLANT GROWTH –PROMOTING RHIZOBACTERIA

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ABSTRACT

The aim of this investigation was to study the effect of adding humic acid and plant growth promoting rhizobacteria (PGPR), as foliar and coating application on plant growth, nodulation status and yield of peanut under field conditions. Results indicate that, using microbial inoculation with *Brady rhizobium* as single inoculation and coinoculation with PGPR (*Azospirillum sp.+ Bacillus polymyxa+ Serratia marcescens)* as mixed inocul, conjugated with humic acid at two rates 3L/fed and 5L/fed. signicantly increased shoot dry weight, nodulation status and yield of peanut plant under coating and foliar applications. Incorporation of humic acid as foliar and coating application led to highest significant for all parameters above control. Also, coating application method was higher than foliar application. Shoot mineral content of N,P and K were increased by using coinoculation and addition of humic acid.

Key words: Plant growth –promoting Rhizobacteria, nodulation , coating and foliar application.

ملخص البحث

تهدف الدراسة الى معرفة تأثير اضافة حمض الهيوميك ومنشطات النمو البكتيرى على تحفيز الريزوبيا المتخصصة (البرادى ريزوبيا)واثر ذلك على نمو نبات الفول السودانى وحالة التعقيد والمحصول الكلى وكذلك حالة النبات الغذائية من النيتروجين والفوسفور والبوتاسيوم وكانت الاضافات اما رشا على النباتات او تغليف مع البذرة.

اظهرت النتائج ان اضافة حمض الهيوميك بمعدل ٣و ٥ لتر/فدان ادى الى زيادة معنوية فى كل من الوزن الجاف و الانتاجية وكذلك محتوى العناصر الغذائية , وكذلك ادى اضافة منشطات النمو الى زيادة معنوية فى النمو وحالة التعقيد ومحتوى العناصر الغذائية لنبات الفول السودانى , وكانت افضل المعاملات فى الانتاجية هة معاملة الخليط بين حمض الهيوميك ومنشطات النمو البكتيرى , وتميزت معاملة الرش عن الاضافة الارضية .

الكلمات الدالة : نمو النبات - تحجيم الجذور البكتيرية، التعقيم، الطلاء و التطبيق الورقي.

1. INTRODUCTION

Groundnut or Peanut (*Arachis hypogaea* L.*)* is considered to be one of the most important edible legume crops in Egypt, due to its seeds has high nutritive value for human and the produced cake as well as the green leafy hay for livestock. In addition, its seeds oil is very important for industrial purposes. Groundnut seeds contain about 50% oil, 25-30% protein, 20% carbohydrates and 5% fiber **(Fageria *et al*., 1997)**. Due to the intensive farming, Egypt is known as a heavy consumer of chemical fertilizers. This intensive farming has caused negative effects on soil environment over the past decades, i.e. loss of soil organic matter, soil erosion and water pollution. The use of chemical fertilizers has been doubled during the last two decades. Thus the coincident application of organic manures and bio-fertilizers is frequently recommended, firstly for improving biological, physical and chemical properties of soil and secondary to get high and clean agricultural yield produced free from undesirable high doses of heavy metals and other pollutants.

Application of mineral N fertilizers has increased in the world agriculture, but too much of such nitrogen form can lead to a terrestrial and aquatic pollution and needs to be reduced (**Mengel *et al*., 2001**). Biofertilizers are alternatives as to increase soil productivity and improve plant growth in a sustainable agriculture regime. Biological nitrogen fixation (BNF) is the most important biochemical reaction for life on earth (**Bohlool *et al*., 1992**).

Plant growth promoting rhizobacteria (PGPR) have the ability to enhance plant growth either directly, by phytohormons production, N_2-fixation and siderophores production…ect., or indirectly, through biological control of pathogens or induction of host defense mechanisms **(Dey et al 2004; Zahir et al 2004 and Verma et al 2010)**.

Enhancement of nodulation and biological nitrogen fixation by co-inoculation legumes with PGPR are becoming a practical way to improve nitrogen availability in sustainable agricultural production system **(Bai et al 2002 and Abdel-Wahab et al 2008)**. The most commonly implicated mode to stimulate of legume-*Rhizobium* sympiosis is phytohormones inducing stimulation of root growth, to provide more sites for rhizobial infection and nodulation **(Vessey and Buss, 2002).**

One of the used organicmineral fertilizers is humic acid. Humic acid is one of the major components of humic substances. Humic matter is formed through the chemical and biological humification of plant and animal matter and through the biological activities of microorganisms (**Anonymous, 2010**). Under water stress, foliar fertilization with humic molecules increased leaf water retention and the photosynthetic and antioxidant metabolism (**Fu Jiu, 1995**). Several researchers, , **Neri *et al*.(2002) and El-Desuki (2004**) concluded that humic acid as foliar sprays enhanced growth nutrient uptake and yield and improved the quality of the production of some crops, this may be decrease the N,P,K applied as soil application which decrease pollution and costs.

Therefore, the objective of this work is to investigate the effect of organic and bio-fertilization on productivity and quality of groundnut seeds, improvement of physical and chemical properties of new reclaimed soils.

2. MATERIAL AND METHODS

2.1. MICROORGANISMS USED

Bradyrhizobium spp. (strain USDA 3456) and mixed of PGPR (*Azospirillum* sp.+ *Bacillus polymyxa*+ *Serratia marcescens*) were kindly obtained from the Biofertilizers Production Unit, Agric. Microbiology. Dept., Soils, Water and Environ. Res. Inst. (SWERI), Giza, Egypt.

2.2. HUMIC ACID

Humic acid, applied at two rates of 3L and 5L/fad. Application the coating with sowing but the foliar at three equal doses applied at 30, 45, 60 days from sowing as a foliar application.

2.3. FIELD EXPERIMENT

Field experiment was conducted in a private farm at Meat Fares, perket El-sabaa ,Menoufia Governorate , Egypt in a sandy loam that contain ,68.9 sand ,24.85 silt and 6.25 clay, pH was 7.13 in soil pastand E.C 0.36 dSm^{-1} ,during summer season 2016 to study the effect of inoculation with *Bradyrhizobium* individually or mixed with (PGPR) and or humic acid at two method , coating and foliar applications, on growth, nodulation status, and productivity of peanut (groundnut) as well as mineral content in plant tissue. Groundnut seeds variety

(Ismaeilia 1) were kindly provided by the Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

A sample weighing 0.2 g of the dried fine materials of peanut shoots were digested with a mixture of 10 ml concentrated H_2SO_4 and $HClO_4$(at a ratio 3:1), on a sand hot plate (at approximately 270 ^oC), until the digest become clear. The digest was then diluted to 100 ml with distilled water. The contents of N, P & K (%) in the diluted digest were determined following the methods stated by (**Cottenie *et al.,* 1982**). The data was later statistically analyzed, as "LSD" (Gomez and Gomez, 1984).

3. RESULT AND DISCUSSION

3.1. SHOOT DRY WEIGHT

Data in Table (1) indicate that using microbial inoculation with *Brady rhizobium* as single inoculation and coinoculation with PGPR (*Azospirillum sp.+ Bacillus polymyxa+ Serratia marcescens)* as mixed incula, humic acid at two rates 3L/fed and 5L/fed. signicantly increased shoot dry weight of peanut plant under coating and foliar applications. Also, coating application was more higher than foliar application when gave the main 18.87 g compared foliar which gave 17.23 g at 45 day after planting . Also, the date in Table, 1 declared that, incorporation of humic acid improvement the growth of shoot and increased shoot dry weight at 45and 75 day after sowing.

Table (1) Effect of microbial inoculation and humic acid on shoot dry weight of peanut.

Treatment	After 45 day from planting			After 75 from planting		
	Coting	Foliar	Mean	Coting	Foliar	Mean
T1	13.59	12.45	13.02	15.40	14.15	14.77
T2	19.56	18.20	18.88	21.90	20.08	20.99
T3	17.48	15.80	16.64	18.10	17.55	17.82
T4	16.48	14.83	15.65	19.30	17.97	18.63
T5	18.61	17.05	17.83	23.00	21.18	22.09
T6	21.87	20.05	20.96	25.90	23.56	24.73
T7	24.47	22.25	23.36	28.40	26.09	27.24
Mean	18.87	17.23	-	21.73	20.08	-
L.S.D. at 5%	0.278	0.792	-	1.45	1.13	-

T1: Control, T2: Recommended dose of mineral fertilizers, T *3: Brady rhizobium ,* T4:Br.+HA3L, T 5: Br,+5l ,

T 6: Br+ PGPR and T7: Mixed of all

It was also observed that, the mixed of all treatment (T7) gave the extra increases in shoot dry weights under two methods applications at 45and 75 day after planting when gave 24.47, 22.25, 28.40 and 27.24, respectively. These increases in shoot dry weights might be due to increase in vegetative growth, which resulted in improvement the nutrient uptake with adding microbial inoculation and humic acid. Hence, the inoculation of Brady rhizobium and PGPR save N2-Fixation, P solubilization, plant growth regulators such as phytohormons and vitamins, and this growth regulators influence plant growth i. e. increased root surface area, respiration rate and metabolism improving mineral and water uptake. These results led to increase in shoot dry weights of plant. This results agreement with those recorded by **El-Howeity (2004).** Also, **Stockwell and Stack (2007)**and **Sindhu *et al.* (2009)** mentioned that, microorganisms inhabiting rhizosphere of legumes may benefit plants in a variety of ways, like increased recycling, mineralization and uptake of nutrients, synthesizing vitamins, amino acids, auxins, gibberlins and plant growth regulating substances, reducing metal toxicity (bioremediation) in contaminated soils, antagonism with potential plant pathogens through competition and development of amensal relationships based on production of antibiotics, siderophores, and/or hydrolytic enzymes.

3.2. NODULATION STATUS

Data presented in Table (2) demonstrated that, the inoculation with specific rhizobia with *Brady rhizobium* significantly enhanced nodulation status , number and weight of nodules/plant at any application methods , increases in number of nodules were 124.66 % and 144.15% above control. Also, increases in weight of nodules were 115.82% and 119.42% above control, respectively. However, Coating method was more effective than foliar application method with number and weight of nodules, when gave 66.23, 313.59, 55.90 and 304.16, respectively. Likewise, coinoculation with *Brady rhizobium* and PGPR was more effective than single inoculation or humic acid addition. So, the application of all treatment T7 led to significant increases and scored higher increases and was more effective compared to other treatments. Humic and PGPR application stimulating legume –rhizobia symbiosis. Since, rhizobium infection tack place by the formation of infection threads in the root hair (**Gage and Margolin 2000),** the stimulation of a greater infect able root hair cells, may provide more sites for infection and nodulation.

Table (2) Effect of microbial inoculation and humic acid application on nodulation status

Treatment	Number of nodules/plant			Weight of nodules /plant(g)		
	Coting	Foliar	Mean	Coting	Foliar	Mean
T1	27.00	19.66	23.33	153.26	151.20	152.23
T2	13.66	16.00	14.83	144.33	138.20	141.26
T3	60.66	48.00	54.33	326.46	333.76	330.11
T4	70.33	64.00	67.16	361.50	337.97	349.73
T5	82.33	75.00	78.66	373.86	372.20	373.03
T6	93.33	84.33	88.83	406.06	393.50	399.78
T7	116.33	84.32	100.32	429.63	407.16	418.39
Mean	66.23	55.90	-	313.59	304.16	-
L.S.D. at 5%	5.49	9.51	-	33.66	13.72	-

T1: Control, T2: Recommended dose of mineral fertilizers, T *3: Brady rhizobium ,* T4:Br.+HA3L, T 5: Br,+5l ,

T 6: Br+ PGPR and T7: Mixed of all.

3.3. YIELD PRODUCTIVITY

Data presented in Table (3) showed the effect of the incorporation biofertilizers and humic acid as coating or foliar application on peanut yield under field conditions. Results indicated that, foliar application was more effective with peanut yield and the weight of 100 seeds but the increases were slight. Irrespective of the adding biofertilizers , inoculation with *Brady rhizobium* plus half doses of nitrogen fertilizer led to significantly increases in yield and weight of 100 seed yields, when gave increases reached to 33.30 and 6.48% above control for the mean yield and weight of 100 seed yield. Increases were more pronounced by using coating application than using foliar application, when gave increases reached with coating (33.59 & 7.53%) and reached with foliar (32.99& 5.44 %) above control for yield and weight of 100 seed yield, respectively.

Table (3) Effect microbial inoculation and humic acid on peanut yield

Treatment	Yield (ton/fed.)			Weight of 100 seeds			Shelling %		
	Coting	Foliar	Mean	Coting	Foliar	Mean	Coting	Foliar	Mean
T1	10.33	10.70	10.51	69.93	71.60	70.76	66.40	67.20	66.80
T2	12.30	12.43	12.36	72.76	73.26	73.01	67.04	67.29	67.16
T3	13.80	14.23	14.01	75.20	75.50	75.35	67.05	67.61	67.33
T4	15.06	15.10	15.08	78.43	81.53	79.98	68.26	68.93	68.59
T5	18.10	18.66	18.38	82.36	80.67	79.61	69.13	69.12	69.12
T6	19.23	20.33	19.78	78.56	83.13	82.74	70.23	69.91	70.00
T7	18.20	18.90	18.55	78.05	78.91	78.48	72.98	72.97	72.90
Mean	15.29	15.80	-	76.47	77.80	-	68.73	69.07	-
L.S.D. at 5%	0.566	0.374	-	1.27	0.52	-	0.3	0.169	-

T1: Control, T2: Recommended dose of mineral fertilizers, T *3: Brady rhizobium ,* T4:Br.+HA3L, T 5: Br,+5l ,

T 6: Br+ PGPR and T7: Mixed of all.

Also, incorporation of humic acid at rate 3and 5l/fed. led to more increases in yield and weight of 100 seed yield over control and above single *Brady rhizobium*, increases with adding humic conjugated with rhizobia reached to (43.48 &12.90%) and (74.88 & 12.50%) above control for yield and weight of 100 seed yield with adding 3 and 5L/fed , respectively.

3.4. TOTAL CONTENTS OF N, P AND K IN PEANUT SHOOT :

Data presented in Tables (4and 5) showed the total contents of N,P and K in groundnut shoots at 45 and 75 days from sowing were greatly affected by adding treatment of inoculation with Brady rhizobium , Plant growth promoting rhizobacteria (PGPR) and humic acid , foliar or coating application. Results showed that the treatment combination ((Br +50%PK +PGPR +5LH) (T7) was significantly superior effect compared to control and all the other treatment in nitrogen , phosphor and potassium content in shoot at 45 and 75 day from sowing . however, it gave the highest mean 825.3, 133.81 and 735.44 for N,P and K at 45 day , respectively . Likewise, it gave 963.75, 157.12 and 769.7 for N,P and K at 75 day after sowing , respectively. Also, coating application was more effective with N and K compared to foliar application but foliar application was more for phosphor. As well as, using full doses from mineral fertilizer increased the content of N,P and K contents in peanut shoots at 45 and 75 day , when gave the mean value (669.8, 110.55 and 599.43) and (748.70, 125.63 and 654.3) For N,P and K at 45 and 75 day after sowing , respectively.

Table (4) Effect of microbial inoculation and humic acid on mineral contents of shoots at 45 day after sowing .

Treatment	N			P			K		
	Coating	Foliar	Mean	Coating	Foliar	Mean	Coating	Foliar	Mean
T1	402.4	362.2	382.3	53.95	58.94	56.44	330.14	311.23	320.68
T2	696.0	643.7	669.8	107.60	113.50	110.55	618.45	580.42	599.43
T3	545.7	497.9	521.8	76.28	81.89	79.08	493.98	488.23	491.10
T4	527.8	474.3	501.3	74.68	79.65	77.16	476.79	466.95	471.87
T5	622.9	559.1	591.0	91.19	92.42	91.80	546.61	537.30	541.95
T6	755.5	686.2	720.8	106.27	117.39	111.83	645.40	636.76	641.08
T7	870.5	780.2	825.3	129.22	137.65	133.81	753.80	717.09	735.44
Mean	631.6	571.9	-	91.39	97.85	-	552.03	534.00	-
L.S.D. at 5%	20.69	25.34	-	3.05	2.28		11.18	24.99	-

T1: Control, T2: Recommended dose of mineral fertilizers, T *3: Brady rhizobium ,* T4:Br.+HA3L, T 5: Br,+5l ,

T 6: Br+ PGPR and T7: Mixed of all.

Furthermore, inoculation with Br. Plus half dose from mineral fertilizer cannot replace full doses, but humic acid with microbial inoculation as mixture led to higher increases in nitrogen, phosphor and potassium content in peanut shoot at 45 and 75day after sowing.

Table (5): Effect of microbial inoculation and humic acid on mineral contents of shoots at 75 day after sowing.

Treatment	N			P			K		
	Coating	Foliar	Mean	Coating	Foliar	Mean	Coating	Foliar	Mean
T1	456.5	411.0	433.75	61.22	66.72	63.97	374.98	353.8	364.3
T2	787.2	710.2	748.70	120.70	130.57	125.63	689.54	619.19	654.3
T3	569.2	576.9	573.05	79.56	85.06	82.31	515.32	509.34	512.3
T4	625.1	582.2	603.65	87.91	93.09	90.50	561.11	549.46	555.
T5	764.4	694.8	729.60	112.72	114.28	113.50	675.42	664.19	669.
T6	934.1	806.1	870.10	131.05	137.87	134.46	757.99	781.52	769.7
T7	1013.2	914.3	963.75	153.81	160.43	157.12	876.69	834.45	
Mean	735.69	670.8	-	106.71	112.57	-	635.91	611.18	
L.S.D. at 5%	21.18	30.44	-	5.58	3.77	-	8.76	38.61	

The improvement in nutrient uptake by using plant growth promoting rhizobacteria may be attributed to several mechanisms such as fixing atmospheric nitrogen, producing siderophores that chelate iron and make it available to the plant root, solubilizing minerals such as phosphorus and producing hormones and synthesizing some compounds or enzymes that can develop plant growth. Indirect growth stimulation of plants is also connected with protection them against the effects of phytopathogens. Yet, the bacteria compete for space at the root of pathogens, where they produce chelators (so-called siderophores) which are specific for the Fe^{3+} ions. Siderophores have a higher affinity for iron than chelators produced by pathogenic microorganisms present in the rhizosphere. Thus, the Fe^{3+} becomes unavailable for pathogens. PGPR bacteria are also capable of the production of secondary metabolites with antibiotic properties or are antifungal substances, insecticides and immunosuppressants (**Kloepper *et al* 2003, Glick, 2005**). The PGPR include free-living soil bacteria that occur in the root zone and endophytic bacteria, colonizing the root cells. The largest group of PGPR bacteria are *Pseudomonas, Bacillus, Enterobacter*, and *Erwinia*. Indoleacetic acid (IAA) that is produced by the bacteria may enhance the effects of plant auxin and can directly affect root growth by stimulating cell division and elongation of the plant **(Kalitkiewicz and Kepczy nska, 2008**).

Islam et al. (2012) revealed that, the biofertilizer *Azospirillum* strains " BM9" and "BM11" positively affected on N,P and k uptake in rice grain and straw yields as single inoculation , they also, found that the interaction of two strains significantly influenced affected on N,P and K uptake .

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