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SYMBIOTIC NITROGEN FIXATION UNDER INTEGRATED ORGANIC-MINERAL NITROGEN APPLICATION ON PEANUT IN SANDY SOIL

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By

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ABSTRACT

A field experiment was conducted on a sandy soil at Ismailia Agricultural Research Station, Ismailia Governorate, Egypt during the summer season of 2016 to study the influence of organo-mineral fertilizer application on nodulation status, yield and yield components of peanut. Four different types of composts originated from different raw materials (cotton stalks, rice straw and bagasse) were integrated with different doses of mineral nitrogen (0, 25, 50 and 100%). Application of any type of compost resulted in enhancement of the nodulation status, plant growth, peanut yield and its components. However, application of rice straw and cotton stalk composts attain the highest values of all the studied parameters. Moreover, the synergy between 50% mineral-N and 50% from any type of compost was superior in all the studied parameters. These fertilization patterns may be considered the most effective strategy for reducing chemical fertilizer, conserving soil fertility and supporting the sustainable agriculture system under sandy soil conditions for producing peanut.

Key words: *peanut, nitrogen fixation, organo-mineral n-fertilizer.*

1. INTRODUCTION

Peanut (*Arachis hypogaea* L.) is considered one of the most important sources of edible oil in the world. The seeds have high nutritive value for human consumption and green leaves are used as hay for livestock (Abdalla *et al.*, 2009). Peanut seeds contain high oil content (50%), which is utilized in different industries, in addition to 26-28 % protein, 20% carbohydrates and 5% fiber (Fageria, *et al.* 1997). The cultivated area of peanut in Egypt during 2011-2012 season was about 62.000 hectares (FAO, 2013). Recently, this crop has been given a great attention due to its suitability for growth in the newly reclaimed sandy soil areas in Egypt.

Most of the newly cultivated area in Egypt is sandy soils which require high rates of chemical fertilizers, aiming to maximize seed yield for human feeding and straw yield to be used in animal feeding and deficient in organic matter as well (Abdel-Wahab *et al.*, 2003). Organic materials including compost are considered the vital soil amendment, as a source of major and minor nutrients, and as a slow release fertilizer (Hoitink and Grebus, 1994).

Nutrient balance is the key component to increase crop yields. Nutrient mining from the soil, deteriorated crop productivity and ultimately soil fertility could be compremized resulted by excess and imbalanced usage of nutrients. Replacement of these nutrients through organics and in combination with organic (organic manures, crop residues and biofertilizers) and inorganic inputs has a direct impact on soil fertility and crop productivity (Datt *et al.*, 2013).

Due to the intensive farming, Egypt is known as a heavy consumer of chemical fertilizers. The immediate application of organic manures and bio-fertilizers is frequently recommended firstly for improving biological, physical and chemical properties of soil, and to get high yield and clean agricultural produce free from undesirable heavy metals and other pollutants (Mahrous *et al.*, 2015).

However, integrated application is needed as alternate nutrient sources for sustaining the desired crop productivity. Tiwari (2002), Doran (1995) and Datt *et al.* (2013) defined the integrated farming system as an agricultural system conceived to have the least impact on the environment.

Shehata (2001) studied the independent application and the combined effects of composts prepared from four organic wastes namely, water hyacinth, pea, orange and chicken manure on squash growth, nutrient contents and fruit yield. He found that the application of compost, which contains the four wastes significantly increased all growth parameters, *i.e.* vine length, the number of leaves, fresh and dry weight of branches and leaves and fruit yield. Chemical analysis of squash fruits revealed beneficial increase in N, P and K due to organic manure application.

On the other hand, many researchers reported positive effects on crop yield following the combined application of compost and inorganic fertilizer (Keeling *et al.*, 2003 and Abdel-Wahab, 2008). There are several explanations for the synergy between compost and fertilizer including alterations of soil water characteristics and mycorrhizal associations (McCallum, 2000). Alternative explanation involve the chemistry of the composts themselves, especially the water soluble fraction, where water extractable humic substances are found in soils and arise through bacterial and fungal action on organic matter over many months and these have been shown repeatedly to be beneficial to plant growth (Ayuso *et al.*, 1996 and Chen *et al.*, 1999).

Symbiotic nitrogen fixation by legumes plays an important role in sustaining crop productivity and maintaining the fertility of the semi-arid lands (Desoky *et al.*, 2011). Vessey and Buss (2002) reported that the most commonly implicated role to stimulate legume-*Rhizobium* symbiosis in photohormones inducing stimulation of root growth, to provide more sites for rhizobia infection and nodulation. Mahrous *et al.* (2015) found that the un-inoculated peanut had the least nodulation status and nitrogenase activity. In thies case, inoculation of peanut seeds with *Bradyrhizobium* exerted considerable improvement in the number and mass of root nodules, and increased the rate of acetylene reduction and all growth characters. In this respect, Sulfab *et al.* (2011), showed that either organic manure alone or with *Rhizobium* inoculation plus 20 kg N/ha significantly increased groundnuts nodulation and early pod formation over the control. Increasing groundnut yield and its components by using the integrated action of both bio-organic and chemical fertilizers were reported by many researchers. Nitrogen at the rate of 20 kg N/ha *via* inoculation with rhizobia coupled with either manures resulted in a significant increment in shoot and root dry weights compared to the control. These treatments also influenced groundnuts shoot N and P accumulation and resulted in significantly the highest pod and straw yields over all other treatments under irrigated and rain fed conditions (Mahrous *et al.*, 2015).

Therefore, in the light of the abovementioned review, the present study aimed to investigate the effect of four types of enriched compost integrated with different levels of inorganic nitrogen fertilizer on symbiotic nitrogen fixation of peanut under sandy soil conditions .

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Organic fertilizer

Composts were supplemented from Soil, Water and Environment Research Institute (SWERI), ARC, Giza, Egypt. The composts were prepared from cotton stalks, rice straw, bagasse and their mixture as primary materials as described in Owis *et al.* (2016). Farmyard manure, rock phosphate, feldspar, bentonite, urea and elemental sulfur at rates of 10, 5, 5, 10, 1 and 1% w/w, respectively, as well as vinasse solution and lignocellulytic inoculant (mixture of *Trichoderma harzianumand, Trichoderma viridi*) at the rates of 1% v/w, respectively, were added as bulking agents and/or amendments. After three months of composting, all heaps were enriched with a mixture of rhizobateria inoculant (*Bacillus polymyxa, Serratia mercescens* and *Pseudomonas fluorescens*) and left for curing 30 days. The main physico-chemical characteristics of the four types of compost are shown in Table (1).

2.1.2. Inorganic fertilizers.

Mineral nitrogen fertilizer was added as ammonium sulfate (20.5% N). All treatments received superphosphate (15.5% P_2O_5) and

Table (1): Physico-chemical characteristics as well as humification and some maturity indices of the used compost.

Character \ Compost type	Cotton stalk	Rice straw	Bagasse	Mixture
Water holding capacity (%)	223.3	141.5	152.6	167.5
Bulk density (kg/m^3)	410.0	600.0	300.0	390.0
pH (1 : 10 extract)	7.95	6.77	6.41	7.12
E.C (dS/m)	4.28	5.53	6.32	5.30
Organic carbon (%)	22.85	23.60	29.40	27.25
Organic matter (%)	39.3	40.59	50.56	48.50
Total-N (%)	1.44	1.42	1.49	1.37
Total-P (%)	0.60	0.46	0.61	0.60
Total-K (%)	2.18	1.60	1.81	1.72
C/N ratio	15.86	16.61	19.73	19.89
N-NO^-_3 (mg/kg)	391.7	371.0	431.9	425.2
N-NH^+_4 (mg/kg)	465.1	441.0	506.0	570.0
Total soluble-N (mg/kg)	757.8	712.0	937.9	995.2
Available-P (mg/kg)	361.5	311.0	316.5	302.8
Available-K (mg/kg)	715.8	613.5	825.4	714.5
E_4/E_6 ratio	2.32	2.54	1.97	2.12
NH^+_4/NO^-_3	1.18	1.18	1.17	1.33
DHA (mg TPF/100g)	86.5	89.8	91.7	90.4
GI for Cress	92.8	79.5	85.0	79.8
GI for Barley	112.2	89.7	111.6	98.6

potassium sulfate (48% K_2O) at the rates of 200 kg and 50 kg/fed, respectively.

2.1.3. Plant material

Peanut (*Arachis hypogaea*), cv., Giza 5, was kindly provided from the Field Crop Research Institute, Agriculture Research Center (ARC), Giza, Egypt.

2.1.4. Preparation of *Rhizobium* inoculum.

Peanut seeds were inoculated with *Bradyrhizobium* sp., which was kindly provided from Biofertilizers Production Unit, Agric. Microbiology Dep., Soils, Water and Environment Research Institute (SWERI), ARC, Giza, Egypt, through using solid inoculant at the rate of 300 g/50 kg seeds with 16% Arabic gum solution as sticking agent.

2.1.5. Field experiment and treatment

Field experiment was conducted during the summer season of 2016 on sandy soil at Ismailia Agricultural Research Station, Ismailia Governorate, Egypt to study the influence of different types of composts in synergy with different levels of inorganic N-fertilizer on nodulation, growth, yield and yield components. The main physical, chemical and biological properties of the experimental soil are shown in Table (2). A randomized complete block design with three replicates was used. Irrigation was done using sprinkle system. The combinations between compost types and mineral fertilizer as rates of organic inputs were developed based on its inorganic N concentration as shown in Table (3).

Five guarded plants were taken from each plot at 75 days after sowing to determine nodulation status, shoot dry weight and its total N-content. At harvesting time, ten guarded plants were taken from each plot to determine yield components, namely pod number/plant, 100-seed weight and crude protein percentage. Plants in the middle three ridges of each plot (3 m^2 area) were harvested to determine the yield of pods, seeds and straw (kg/fed).

2.2. Methods of analysis

Soil and compost characteristics were determined according to Piper (1950) and page *et al.* (1982). Total-N content and crude protein content in plant tissues and seeds were assayed according to Page *et al.* (1982). Nitrogenase enzyme activity in fresh roots was measured using acetylene reduction assay as described by Hardy *et al.* (1973). All data of plant parameters were statistically analyzed according to Snedecor and Cochran (1980).

Table (2): The main physical, chemical and biological properties of the experimental soil.

Character	Value
Particle size distribution:	
Sand (%)	90.30
Silt (%)	4.90
Clay (%)	4.80
Texture grade	Sandy
$CaCO_3$	1.72
S.P (%)	21.10
pH (soil paste)	7.57
E.C. (dS/m at 25°C)	0.32
Soluble cations (meq/L):	
Ca^{++}	0.84
Mg^{++}	0.52
K^{+}	1.51
Na^{+}	0.15
Soluble anions (meq/L):	
CO^{--}_3	0.00
HCO^{-}_3	1.72
Cl^{-}	0.68
SO^{--}_4	0.62
Organic matter (%)	0.53
Total-N (%)	0.03
Available-P (mg/kg))	5.95
Available -K (mg/kg)	58.7
Available -Fe (mg/kg)	2.30
Available -Mn (mg/kg)	0.50
Available -Zn (mg/kg)	0.60
Log No. of bacteria	4.86
Log No. of fungi	3.56
Log No. of actinomycetes	4.11
Dehydrogenase activity (mg TPF/100g)	8.70

3. RESULTS AND DISCUSSION

3.1. Vegetative stage

Vegetative stage was evaluated *via* nodulation status, shoot biomass and their N-content of peanut plants grown on sandy soil 75 days after sowing. Fig. (1) demonstrated that mineral N fertilizer integrated with organic N led to significant increase in nodulation status which is expressed by nodule number, nodule dry weight and nitrogenase enzyme activity in comparison to sole application of mineral nitrogen fertilizer. However, the application of zero mineral N and 100% of any type of compost surpassed the other treatments for nodule number, particularly zero N and 100% mixture of the three types of compost. The peanut plants fertilized with 50% mineral N and 50% compost gave the highest nodule dry weight and nitrogenase enzyme activity, especially with rice straw compost. While, the application of 100% mineral N fertilizer showed the least effect on nodulation status, where the least values of nodule number, nodule dry weight and nitrogenase enzyme activity were recorded.

The positive effect of compost combined with N fertilizer on nodulation status may be attributed to the vital role of active organic compounds in improving the physical, chemical and biological properties of sandy soil such as increasing of nutrients availability, microbial activity in rhizosphere and enhancing the root proliferation and consequently boosting the intact nodulation pattern. These results were in harmony with (Abdel-Wahab *et al.*, 2003 ; Abdel –Hafez and Abo El Soud, 2007).

Table (3): The combinations between compost types and mineral fertilizer.

Combinations Compost : Mineral	Mineral fertilizer (kg/fed)	Compost type (ton/fed)			
		Cotton stalks	Rice straw	Bagasse	Mixture
100% : zero	-	2.78	2.81	2.68	2.91
25% : 75%	30	0.69	0.70	0.67	0.73
50% : 50%	20	1.39	1.41	1.34	1.46
zero : 100%	40	-	-	-	-

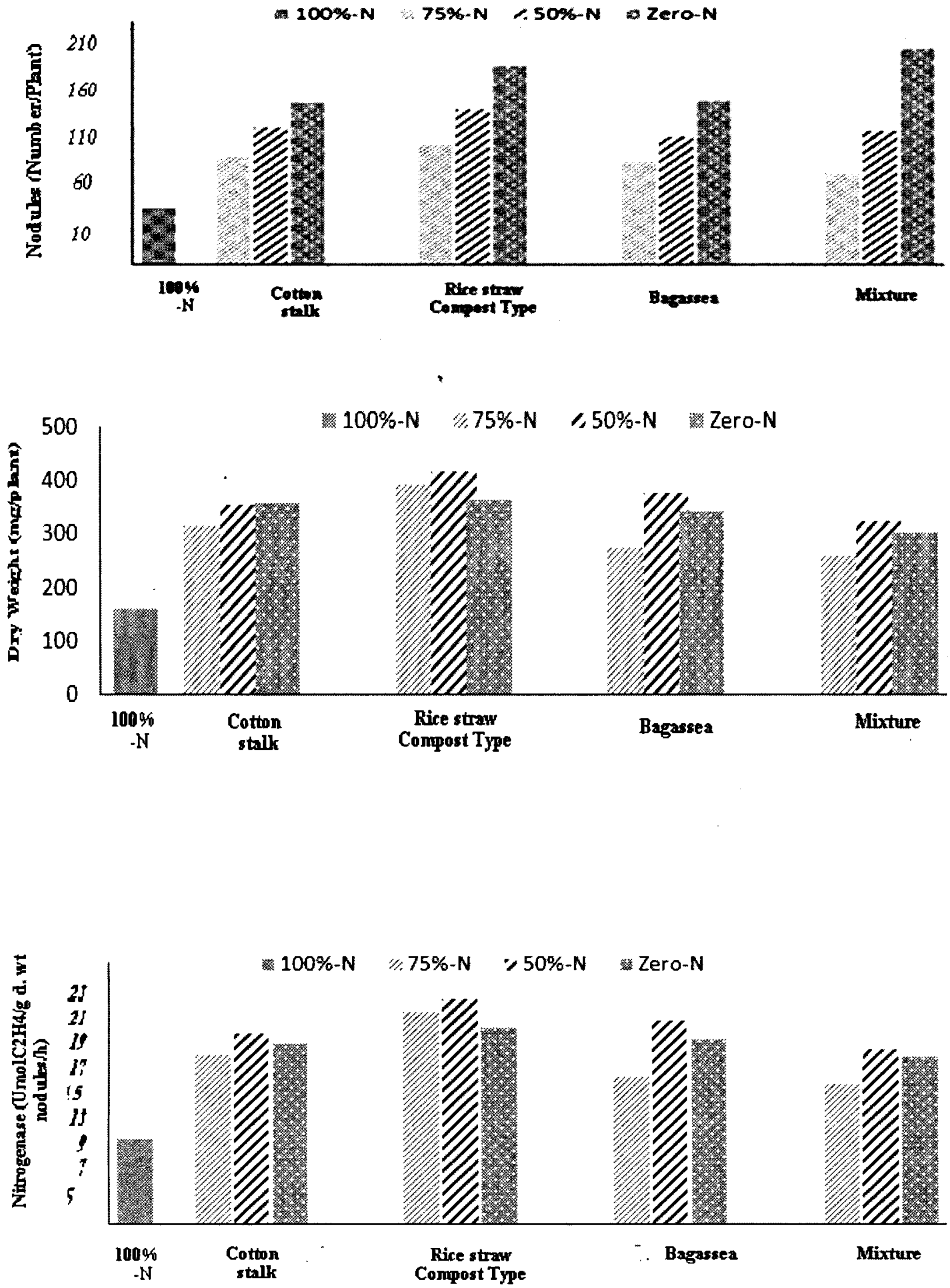

Fig. (1): Effect of organic-mineral nitrogen application on nodules number, dry weight and nitrogenase enzyme activity of peanut.

Also Ibrahim *et al.* (2011) and Mohammadi *et al.* (2011) reported that the positive influence of organic manure application enhances the root growth and the uptake of nutrients, and thus improves the nodulation. This observation is in agreement with other studies where organic matter has been shown to increase the viable number of rhizobia and nodulation of peanut (Basu *et al.*, 2008).

On the other hand, negative effect of high N rate fertilization on nodulation status may be due to the inhibition of the formation of infection threads or suppression of nitrogen fixation by nodules, (Ahmad, 2013 and Agha *et al.*, 2004).

The application of organic fertilizer rich in nitrate did not suppress nodulation in the experimental sites though this N-rich material with C:N ratio less than 17:1 enhanced mineralization by microorganisms. However, the negative effect of N on nodulation was not observed. Lack of inhibition effect of N might be because of the attenuated effect of other essential nutrients found in the organic input (Burgos *et al.*, 2006). Wu and Arima (1992) reported that N applied with other nutrients increased nodulation, whereas N applied alone reduced the nodule formation. This result suggests that application of medium rich N of organic fertilizer is essential to enhance nodulation in degraded and low fertile sand soils.

Concerning the effect of compost types or/and mineral N-fertilizer on shoot dry matter and their N-content it is clear that top-dressing with any compost type resulted in a significant increase in the plant vigor and N-content of peanut in comparison to treatments received mineral-N only, except for bagasse compost type with zero and 100% where shoot dry weight decreased (Figs. 2 and 3).

However, application of rice straw and cotton stalk composts resulted in the highest shoot biomass and its N-content compared with the other compost types, particularly, when combined with 50% from mineral-N. The positive effect of organic substances on developing the plant vigor and accumulating the N-content in their tissues may be attributed to increase the availability and translocation of nutrients, besides the high biological properties of the used composts, which contained the beneficial rhizobacteria leading to boost the promotive effect on plant vigor as confermid by many workers (Zhang *et al.,* 1998; Lalande *et al.,* 2000; El-Tahlawy, 2006 and Abdel-Wahab *et al.,* 2009). Ojo *et al.* (2014) and Smith *et al.* (2015) recommended using various organo-mineral fertilizer composts as partial substitutes to mineral fertilizers in arid and semi-arid regions as

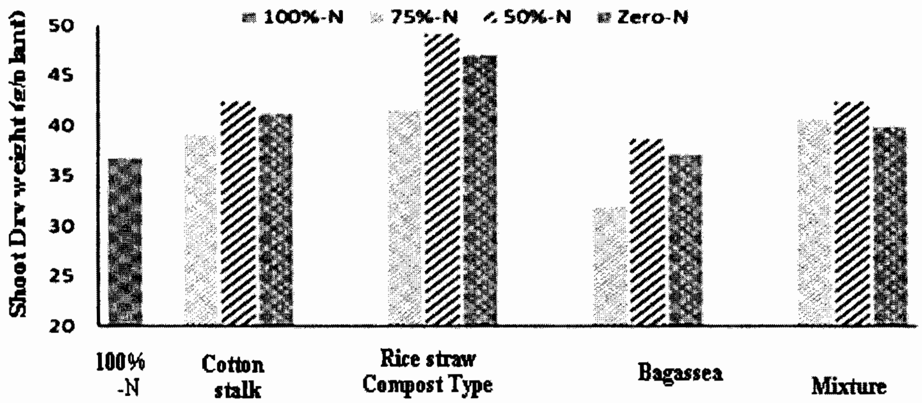

Fig. (2): Effect of organic-mineral nitrogen application on the dry weight of peanut shoot.

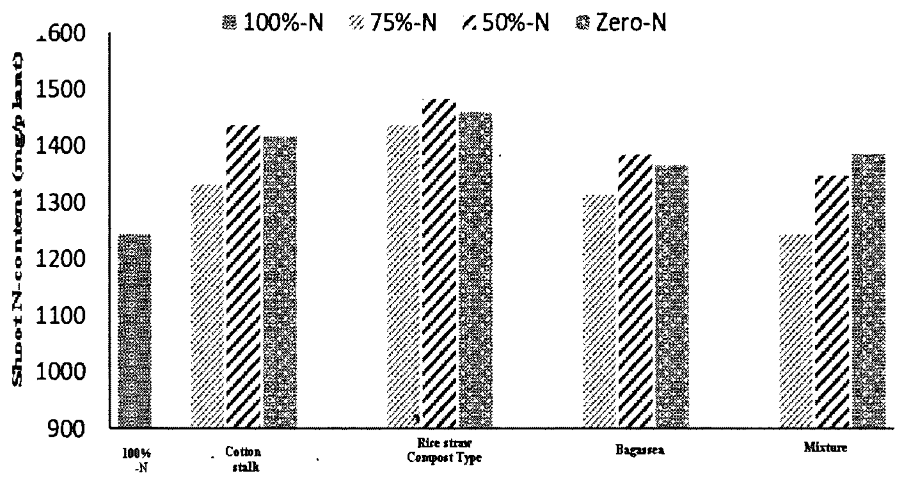

Fig. (3): Effect of organic-mineral nitrogen application on N-content of peanut shoots.

source of nutrients, because these soils inherently have low organic matter and low mineralization due to high alkalinity and low rains. Because organic manures are characterized by slow release of nutrients, the complementary applications of organic manures with fast nutrient release-characterizing mineral fertilizers have been advocated (Rady *et al.*, 2016). Rady (2011) and Riaz *et al.* (2015), also reported the beneficial effects of this practice in terms of improved crop productivity, soil fertility and sustainability, and balanced plant nutrition.

The improved growth characteristics of common bean plants produced as a result of increased application of organo-mineral fertilizer compost could be attributed to the enhanced decomposition of organic matter and mineralization of nutrients (Abdelhamid *et al.*, 2004 and Ojo *et al.*, 2014).

Various soil-microorganisms may benefit from some components of the organo-mineral fertilizer compost to increase their excretions of vitamins, growth substances, and antibiotics that can further promote plant growth (Osman and Rady, 2012 and Semida *et al.*, 2014).

3.2. Peanut yield and its components

Peanut yield and its components as affected by different types of compost or/and different levels of mineral nitrogen fertilizer are shown in Table (4). Results showed that the peanut yield and its components achieved the highest values as a result of soil manuring with different types of bioenriched compost, particularly rice straw and cotton stalk composts. However, the types of compost existing in blend with mineral fertilizer at equal ratio (50% and 50%), recorded the highest values of peanut yield and its components. For instance, the values attained for seed yield (kg/fed); straw yield (kg/fed), pod number/plant, hundred seed weight (g), seed crude protein and straw crude protein contents (%) as a result of addition of 50% rice straw compost in synergy with 50% mineral-N were 989.53, 1366.2, 27, 27.93, 87.92 and 11.52. The corresponding values recorded in the case synergy of 50% cotton stalk compost with 50% mineral-N fertilizer were 980.0, 1352.5, 25, 86.74, 27.27 and 10.38, respectively.

This shows the importance of organic fertilizer application to increase yield of peanut. This pronounced effect of organic inputs on final yield of peanut could be associated with the mineralization and releasing of nutrients from organic inputs at the late stage of the plants. This is in harmony with Khater *et al.* (2004) who reported positive benefits for improving soil characteristics under study due to compost amendments application where an improvement in the values of bulk density, hydraulic conductivity, soil consistence, available water content, organic matter

Table (4): Influence of different compost types in synergy with different N-levels on peanut yield and its components.

Treatments	Straw yield (kg/fed)	Pod number/ plant	Pod yield (kg/fed)	Seed yield (kg/fed)	100-seed weight (g)	Crude protein (%)	
						Straw	Seed
100% N (40 kg N/fed) fertilizer							
Without compost	1198.8	22	1118.50	911.00	74.55	9.83	26.54
75% N (30 kg N/fed) fertilizer + 25% compost							
Cotton stalk compost	1187.3	24	1119.07	911.70	81.80	9.91	26.34
Rice straw compost	1199.9	24	1133.73	919.13	83.00	10.17	27.86
Bagasse compost	1259.5	22	1131.63	914.68	80.65	10.15	27.17
Mixture compost	1261.9	23	1110.67	923.67	82.42	10.08	26.88
50% N (20 kg N/fed) fertilizer + 50% compost							
Cotton stalk compost	1352.5	25	1183.00	980.00	86.74	10.38	27.27
Rice straw compost	1366.2	27	1198.00	989.53	87.92	11.52	27.93
Bagasse compost	1292.0	25	1174.21	963.12	85.18	11.10	27.42
Mixture compost	1309.3	26	1178.67	956.67	86.38	11.25	26.95
Zero N fertilizer + 100% compost							
Cotton stalk compost	1249.6	23	1178.55	957.19	85.52	11.10	27.62
Rice straw compost	1258.1	24	1187.17	941.13	87.00	10.13	26.76
Bagasse compost	1231.5	23	1162.78	939.77	85.11	10.17	27.11
Mixture compost	1268.4	24	1159.18	951.59	86.72	11.13	26.81
LSD 0.05	7.6	1.5	5.9	5.6	3.2	0.4	1.2

content and the released content of available nutrient *i.e.* N, P, K and Cu were recorded. Also findings by (Abdel-Wahab *et al.*, 2009), reflected the essential role of distinct bio-organic substances in improving peanut productivity in sandy soil, which might be elucidated by the vital role of bio-organic materials in improving the physical, chemical and biological properties of sandy soil, reflected on plant nutritional status leading to support plant growth and consequently augmentation of peanut yield and its components.

The benefits of organic and inorganic fertilizer application in combination with rhizobia on the agronomic productivity of food legumes have been previously reported in chickpea (Namvar *et al.*, 2013;). Also, Shahzad *et al.* (2013), demonstrated a significant increase in soil nutrients including available P by organic matter application.

The increase in pod and seed yield treated with rice straw might be due to improved soil condition coupled with large surface area of straw at low dosage which promotes faster decay and release of nutrients. This is in agreement with (Jagdev and Singh 2000 and Abdul Aziz *et al.*, 2016), who reported that rice straw had C/N ratio of 1:1, which is a clear indication that the material can decompose rapidly. Its high organic matter and low organic carbon concentration were adequate for the growth and development of groundnut.

From these results, it could be concluded that the synergy between compost enriched with natural amendments and rhizobacteria as half dose of N-fertilizer source and mineral-N fertilizer (as second source) led to increase the growth and productivity of peanut under sandy soil conditions.

This fertilization pattern may be considered the most effective strategy for reducing chemical fertilizer, conserving soil fertility and supporting the sustainable agriculture system under newly reclaimed soils for producing peanut.

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تثبيت النيتروجين تكافليا عند تطبيق التكامل بين النيتروجين العضوي – المعدني في نباتات الفول السوداني في الأراضي الرملية

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ملخص

اجريت تجربة حقلية فى تربة رملية بمحطة الإسماعيلية للبحوث الزراعية بمحافظة الإسماعيلية خلال صيف 2016 لدراسة تأثير تطبيق التكامل بين الأسمدة العضوية والازوتية المعدنية على تكوين العقد البكتيرية ومحصول الفول السوداني. تم استخدام أربعة أنواع مختلفة من السماد العضوي المصنعة من مواد خام مختلفة (حطب القطن، قش الارز، الباجاس ومخلوط من المود السابقة) مع اربع جرعات مختلفة من النيتروجين المعدني (0، 25، 100,50%).

أدى استخدام أي نوع من السماد العضوي إلى تحسين تكوين العقد البكتيرية ونمو النبات وزيادة المحصول وبعض مكوناته فى الفول السوداني. واضافة لذلك، فإن استخدام كمبوست قش الأرز وحطب القطن حقق أعلى القيم لجميع الصفات تحت الدراسة. وقد ادى التكامل بين السماد العضوى و الازوت المعدنى بنسبة 50٪ نيتروجين معدني مع 50% من أي نوع من الأسمدة العضويةالى تفوق في معظم الصفات المدروسة. هذا ويمكن اعتبار استراتجية التكامل بين التسميد العضوي مع المعدني أكثر فعالية في الحد من الأسمدة الكيماوية والحفاظ على خصوبة التربة ودعم نظام الزراعة المستدامة لزراعة الفول السودانى تحت ظروف الاراضي الرملية.

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