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Importance of Bio-Organic Fertilizers on Peanut (*Arachis Hypogaea* L.) Nutrition Following Organic Farming Approach with Application of ^{15}N Isotope Dilution Concept

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FIELD trial was conducted under low fertile sand soil conditions to follow up the role of organic additives with presence and absence of compatible bacterial inoculum in enhancement of peanut growth and yield.Seed yield was positively affected by *Bradyrhizobium* spp. (foreign strain USDA 3456), inoculation that resulted in 5% increases over the un-inoculated plants. Organic additives increased the seed yieldover the un-treated plant by about5%, 16% and 37% for animal manure, leuceana residues and quail feces, respectively.Seeds and straw of inoculated plants accumulated more nitrogen than roots and seed cover. Inoculation has an enhancement effect on N uptake by different plant parts. On overall means basis of N uptake by seeds, comparison between organic additives indicated the superiority of animal manure over both of leuceana residues and quail feces. All organic additives has enhanced N uptake by seeds and straw comparing to the un-treated control. Nitrogen derived from mineral fertilizer seems to be higher in absence of organic manure and tended to decrease with addition of different organic amendments. In the same time, it was higher in case of the un-inoculated plants than the inoculated one. Accordingly, the portion of fertilizer-N remained in soil after harvest was, in general, very low especially under organic amended soil and in absence of bacterial inoculation. It seems that application of organic amendments to substitute mineral fertilizer in combination with bacterial inoculation contributed to minimize N losses from soil media. Nitrogen derived from air by different plant parts on the basis of overall means indicated higher records accounted for 45.22 kg ha^{-1} with treatment leuceana residues followed by quail feces (45.22 kg ha^{-1}) and animal manure (32.89 kg ha^{-1}), respectively but all of them were superior over the un-treated treatment. In this respect, leuceana additive achieved relative increase in Ndfa values by about 92%, 51% and 10% over un-treated control, animal manure and quail feces, respectively.Portion of N derived from organic sources was higher in seeds and straw than roots and seed cover, respectively. Differentiation between the different organic sources was varied according to plant organ. Also, bacterial inoculation reflected an enhancement effect on increasing the availability of N from the different organic sources.

Keywords: *Bradyrhizobium*, Groundnut, Inoculation, Soil manuring, Stable isotope.

Introduction

Groundnut (*Arachis hypogaea* L.), belonging to family Fabaceae, is an important annual legume in the world for oilseed, food, and animal feed. Being a soil nitrogen-fixing crop, it is safe, cheap, and eco-friendly for the soil environment. It is a good source of vegetable oil and protein for humans (Abbas et al. 2018). It is grown on 26.4 million ha worldwide with a total production of36.1 million metric tons, and an average productivity of 1.4 metric tons per hectare (FAO, 2004).

Use of biofertilizers is considered a securing approach to achieve sustainability via organic

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farming as application of beneficial soil microbes as biofertilizers has a wide range of functions in controlling soil health and crop productivity. It is essential to develop effective strategies for better inoculation so that the inoculating bacteria gain the benefit for faster and effective colonization and exhibit higher competitiveness in the inoculated niche. The plant growth-promoting potential of inoculants combined with compatibility and shelf life are the key factors required for efficient colonization and performance under field conditions (Lee et al. 2016).

The effectiveness of biofertilizers for enhancing crop productivity was discussed by Sahu et al. (2018) and they classified beneficial microbes into two classes-plant growth promoters and plant health promoters. The plant growth promoters provides nutrients and growth enhancers to the plant and gives good growth in absence of a pathogen, for example, N_2 fixers, P solubilizers, P mobilizers, plant growth promoters, etc. (Nehra and Choudhary, 2015; Sahu and Brahmaprakash, 2016; Takanashi et al. 2016). In the same time, plant health promoters sustain the plant growth in presence of a pathogen or any abiotic stress. It either blocks the pathogen directly or minimizes deleterious effects of pathogen. Entomopathogenic fungus *(Beauveria bassiana and* Metarhizium anisopliae), *Trichoderma*, nematode-trapping fungi and other biocontrol agents come under this class (Gorzelak et al. 2015; Labroussaa et al. 2016; Qi et al. 2016; Yan et al. 2016). Microbes also alleviate abiotic stresses by inducing/secreting different metabolites in the plants (Tiwari et al. 2011; Pandey et al. 2016).

Application of organic sources increased quality seed production, but highest seed yield and seed quality parameters of peanut were recorded with application of farm yard manure (FYM) at rate 5 ton ha^{-1} in the presence of *Rhizobium* and phosphorus solubilizing microorganism followed by Neem cake applied at rate 1.5 ton ha^{-1} with the same biofertilizers (Panwar et al. 2002). On sandy soil of Egypt, Hellal et al. (2014), reported significant differences with regard to yield parameters (100 seed weight, seed yield and foliage yield) of bean plants. They recorded the highest yield parameters with application of farmyard manure in combination with effective microorganisms (EM) followed town refuse while the lowest value was recorded in the treatment received Biogas manure. Also, significant differences among the treatments were noticed with respect to available NPK and uptake by bean due to manure application. Application of FYM combined with EM recorded the highest available and uptake of NPK and significantly superior over other treatments including control. As reviewed by Basu and Kumar (2020), members of Leguminosae plant family performed symbiotic relation with compatible bacteria of *Rhizobium, Azorhizobium, Bradyrhizobium, Sinorhizobium,* and *Photorhizobium* (Newton, 2000), via development of root nodules formation (Udvardi and Poole, 2013; Fujita et al. 2014).

Legumes and rhizobia together fix atmospheric nitrogen and the symbiosis also have a vital role in improving the organic fertility of soil as well as its economy (Jeffries et al. 2003). Nitrogen is an essential element of agricultural sustainability that involves the effective management of the soil. About 80% of biologically fixed nitrogen comes from syntheses formed between leguminous plants and species of *Rhizobium, Bradyhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium,* and *Allorhizobium* (Vance, 2001). Plants and microbes help each other: plants provide nutrients to the microbes and receive nitrogen in the reduced form from the microorganisms. Legume plants form a specialized atmosphere where rhizobia fix atmospheric nitrogen. These specialized plant structures, known as nodules, are generally established on the roots and at times on the stems of the plant (Kong et al. 2017). Microbes that are tolerant to stress have better nodulation ability and greater ability for nitrogen fixation of legumes to grow and survive under stressed conditions. Rhizobial populations vary in their tolerance to major environmental factors. In addition to nitrogen fixation, these beneficial microorganisms exhibit control activity as well: rhizobia are used as biofertilizers under severe conditions (Shiraishi et al. 2010).

Accordingly, we established a management scenario to achieve the best way for maximizing the groundnut yield without environmental risks and in the same time benefits from the low-cost agriculture approach.

Materials and Methods

Seeds of peanut (*Arachis hypogaea* L. Giza 5) provided by the Legume Crop Department, Institute of Field Crops Research, Agriculture Research Center, Giza, Egypt, were sown at med of May 2018 on sandy soilunder drip irrigation system. Some physical and chemical properties of the experimental soil are presented in Table 1. Experimental site was located at Inshas area belongs to Sharqueia Governorate, Egypt with the latitude and longitude of 30°24` N and 31°35`E, respectively, while the altitude is 20 m above the sea level.Irrigation water requirements for growth season were estimated according to meteorological data using neutron probe. Prior to cultivation, the soil was prepared by adding the recommended rates of phosphorus and potassium fertilizers which thoroughly mixed with the soil. Phosphorus was applied in the form of rock phosphate at rate of 192 kg rock-P ha^{-1}, while potassium was added in the form of potassium sulfate at rate of 120 kg ha^{-1}. Animal manure, leuceana residues and quail feces as organic additives (Table 2) were incorporated into the soil 45 days before sowing to offer good fermentation and breakdown (semi digested state). The quantities of organic additives were estimated according to its nitrogen contents. All organic additives were applied in the presence and absence of *Bradyrhizobium* spp. (foreign strain USDA 3456) which supplied by biofertilizers Production Unit, Soils, Water and Environment Research Institute, ARC, Egypt. Seed inoculation was carried out according to Somasegaran and Hoben (1994). The experimental treatments (organic sources x inoculation) could be described as following:

1. Without organic source (only sand soil with 0.5% N).
2. Animal manure with 1.6% N content was added at rate of 9237 kg ha^{-1} equal to 108 kg N ha^{-1}.

TABLE 1. Some physical and chemical characteristics of the experimental soil

Particle size distribution (%)				Teture class		Bulk density g cm^3		F.C. %		PWP %	
Sand		**Silt**	**Clay**	**Sandy**		**1.37**		**9.2**		**2.1**	
87.6		4.5	7.9								
pH 1:2.5	$CaCO_3$ (g kg^{-1})	OM (g kg^{-1})	EC (dS m^{-1})	Soluble cations (mmol$_c$kg^{-1}soil)				Soluble anions (mmol$_c$ kg^{-1}soil)			
7.4	0.00	3.5	1.67	Ca^{++}	Mg^{++}	Na^+	K^+	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
		3.8	7.5	1.8	3.6	-	4.4	5.9	6.4		

TABLE 2. Some chemical characteristics of organic sources

Value	Organic sources			
	Quail feces	**Leucaena residues**	**Value**	**Animalmanure**
N	4.20	5.3	pH (1:5)	6.68
P	1.16	0.3	EC,dSm^{-1}	13.3
K	1.08	2.7	C/N ratio	26.0
Na	0.52	0.09	OM (%)	39.89
Ca	3.53	0.2	N (%)	1.60
Mg %	1.80	0.7	P (%)	0.53
Fe	0.14	0.2	K (%)	0.507
Cu	0.01	0.01	Total Fe	2730
Zn	0.56	0.3	Total Cu,µg g^{-1}	148.08
Mn	0.44	0.03	Total Mn	130.75
C/N ratio	13:1	18:1	Total Zn	222.58
Free amino acids (ppm)	46.8	52.03		

3. Leuceana residues with 5.3% N were added at rate of 2030 kg ha^{-1} equal to 108 kg N ha^{-1}.
4. Quail feces with 4.2% N were added at rate of 2551 kg ha^{-1} equal to 108 kg N ha^{-1}.
5. Half of seeds of peanut plants were inoculated with Bradyrhizobium spp. and the rest kept without inoculation.

This experiment has 4 organic treatments x 2 inoculation treatments x 3 replicates equal to 24 plots with area of 61.2 m^2 per treatment. Micro-plot with area of 1 m^2 was included to add labeled nitrogen fertilizer in the form of urea enriched with 5% ^{15}N atom excess to follow up the transformation and portions of N derived by peanut plants from the different N sources, e.g. nitrogen derived from fertilizer (Ndff), nitrogen use efficiency (NUE%), nitrogen derived from organic sources (Ndforg), nitrogen derived from air (Ndfa) and those remained in soil after harvest. This labeled fertilizer was added at 50% of the recommended rate in addition to 50% added in organic form as mentioned above. This indirect method helps in differentiation between different N sources and gave an accurate estimation of N gained by plants and in the same time recognizing the best or appropriate N management. At the end of October 2018 (160 days), plants were harvested and air dried then transferred to the lab for preparation to analyses. Plant samples were collected and separated into roots, straw, pods, seedsand seed shell then dried in oven at 70 ^{0}C until constant weight. Dry weight of different plant parts was recorded. Root nodules were also examined.

Laboratory analyses

Soil and organic sources analyses were carried out according to Carter and Gregorich (2008). Plant analyses were carried out as described by Estefan et al. (2013). $^{14}N/^{15}N$ ratio analysis was carried out using NOI-6 PC emission spectrometer. Details of isotope dilution technique are described by IAEA-TEC-DOC no. 14 (2001).

Equations

$$\% Ndff = \frac{^{15}N\% \text{ atom excess of plant}}{^{15}N\% \text{ atom excess of fertilizer}} \times 100$$

Nydff = %Ndff x total N uptake.

$$\% FUE = \frac{Nydff}{\text{Rate of fertilizer applied}} \times 100$$

$$\% Ndfa = \left(1 - \frac{^{15}N\% \text{ atom excess in inoculated plant}}{^{15}N\% \text{ atom excess in un-inoculated plant}}\right) \times 100$$

$$\% Ndforg = \left(1 - \frac{^{15}N\% \text{ atom excess in treated with plant residues}}{^{15}N\% \text{ atom excess in untreated with plant residues}}\right) \times 100$$

$$\% \text{ Fertilizer-N remained in soil} = \frac{^{15}N\%a.e. \text{ in soil}}{^{15}N\%a.e. \text{ in fertilizer added}} \times 100$$

The obtained data were statistically analyzed using SAS, software program, (2002). The Least Significant Deference (L.S.D) at the 0.05 level was detected.

Results and Discussion

Dry matter yields (kg ha^{-1})

Peanut plants treated with different organic sources in presence or absence of bacterial inoculation (Rhizobium) showed different patterns of dry matter of root, straw, pods and seed yield (Fig. 1). In case of untreated control and animal manure additives, root dry matter yield tended to increase with bacterial inoculation over the un-inoculated treatment. Relatively, it increases by about 26% for both treatments. Reversibly, root dry matter yield under quail feces addition was declined by about 23% as affected by bacterial inoculation. There was no significant difference between the root dry matter yield of inoculated and the un-inoculated plants treated with leuceana residues.

On the basis of grand mean, despite of organic additives, bacterial inoculation induced slight increases of root dry matter yield. It relatively accounted by about 5% over the un-inoculated treatment. Despite of inoculation treatment, the organic additives showed higher root dry matter yield than the untreated control. In this regard, based on grand mean, animal manure was superior over leuceana residues and quail feces, respectively. It worthy to mention that the mean of root dry matter yield under quail feces treatment was nearly closed to those detected with the untreated control.

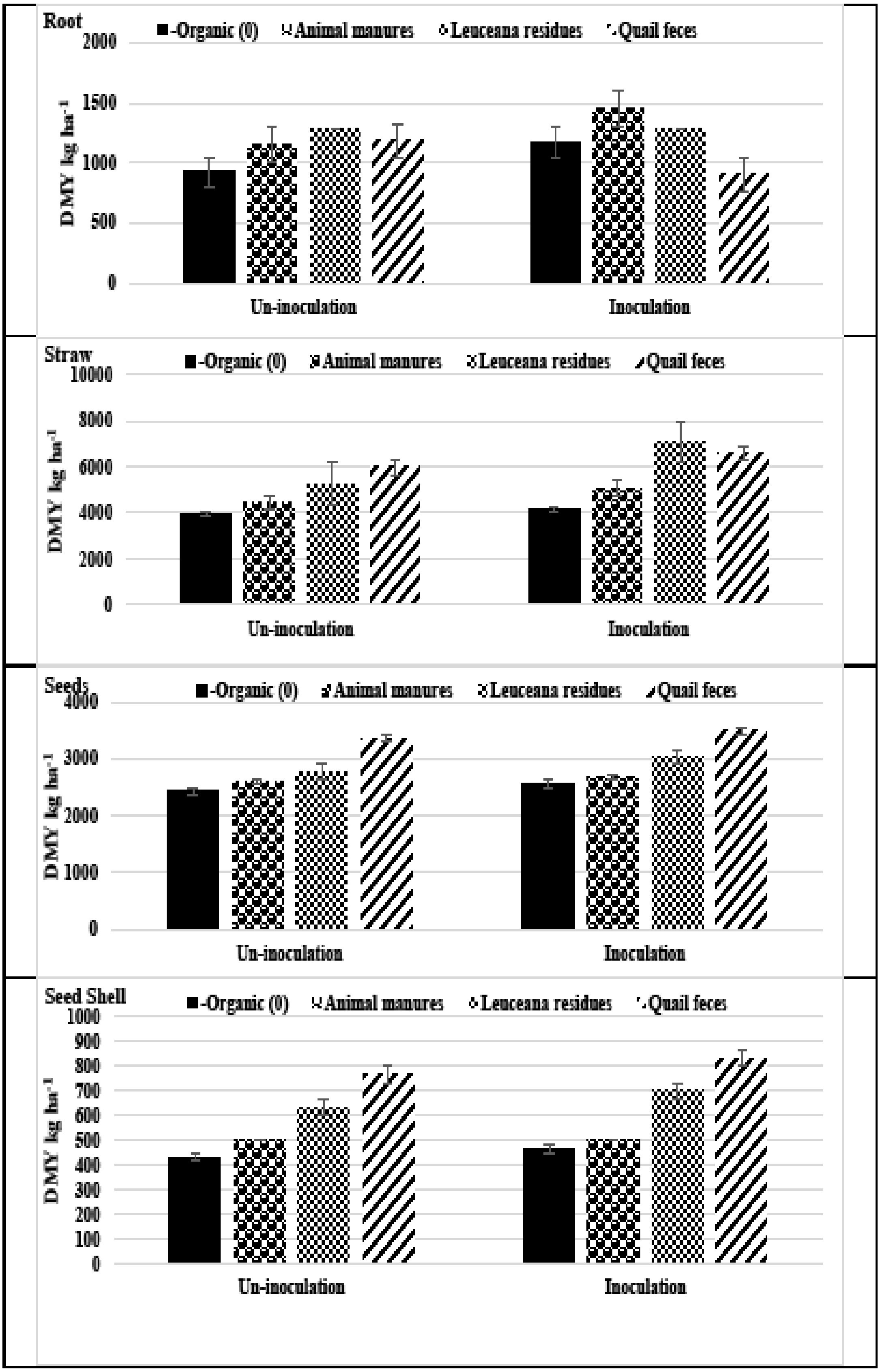

Fig. 1. Effect of Rhizobium inoculation and organic additives on roots, straw, seed shell and seed yield (kg ha^{-1}) of peanut crop grown on sandy soil

Straw dry matter yield was positively significantly responded to bacterial inoculation whereas it enhanced comparing to the un-inoculated one (Table 1). Relatively, it increases by about 5%, 14%, 34% and 10% over the uninoculated plants for untreated, animal manure, leuceana residues and quail feces, respectively. Based on mean, despite of organic treatments, inoculation has enhanced the straw yield by about 16% over the un-inoculated ones.

Dealing with organic additives, straw yield reflected an enhancement comparable to the untreated control. In this respect, despite of inoculation based on mean, quail feces showed the best values among other treatments. Absolutely, the best value of straw yield (7067 kg ha^{-1}) was achieved by leuceana residues applied to the inoculated plants. Generally, organic additives impact, based on mean values, could be ranked as following, quail feces > leuceana residues > animal manure > untreated control.

Seed yield of the un-inoculated plants tended to increase with application of animal manure, leuceana residues and quail feces application as compared to the un-treated plants. Relatively, animal manure, leuceana residues and quail feces resulted in 7%, 14% and 38% increments, respectively over the un-treated control. This result confirmed the superiority of quail feces over leuceana residues and animal manure, respectively. Similar trend, but to higher extent, was observed with inoculated plants indicating the positive significant effect of bacterial inoculation on seed yield. Comparison held between different organic sources added to the inoculated plants indicated that quail feces was the best recording 36% relative increase in seed yield over the un-treated control, while leuceana residues and animal manure induced 18% and 4%, respectively. It seems that quail feces have the ability to enrich soil with nutrients more than others. On overall mean basis, inoculation with Bradyrhizobium resulted in higher seed yield by about 5% over the un-inoculated one. Similarly, quail feces had increased seed yield by about 37% over the un-treated control achieving the highest relative increase among organic sources. It means that plants were much more dependable on organic fertilization rather than bacterial inoculation.

Similar trends with lower extent than other plant parts were noticed with seed shell. Concerning the dry matter yield of plant organs, different organs could be arranged in the following rank: straw > seed > root > seed cover.

In this respect, explanation stated by Hellal et al. (2014) clarified that favorable effects on yield and yield components could be attributed to the stimulation effect of NPK on number and weight of nodules and nitrogen metabolism, which in turn reflected positively on faba bean yield attributes. They added that the increases in yield and its components as a result of application of the farmyard manure over Biogas and town refuse application may be attributed to high content of micronutrients, which might enhance the activity of photosynthesis and protein synthesis in the leaves. This in turn encourages photosynthetic process apparatus. The elemental composition of the organic manure applied especially their content of N, P, K, Fe, Mn, Zn and Cu may account for such finding. On line, beneficial microorganisms in bio-fertilizers found to act as accelerators for improving plant growth and protect plants from pests and diseases (El-Yazeid et al. 2007). In consistent, under sandy clay low fertile soil, nutritional imbalance and inappropriate agronomic practices conditions, Hasan and Bin-Sahid (2016), recorded the highest yields of groundnut inoculated with *Rhizobium* in combination with P and N fertilizers. Similarly, Didagbé et al.(2014), recorded positive significant effect of different *Bradyrhizobium* strains inoculation combined with P fertilizer on growth parameters of groundnut grown on sandy loam and loamy sand poor fertile soils in Benin. Alfandi et al. (2019) suggested the use of rhizobium inoculums and liquid organic fertilizer for increasing seed yield and growth traits of peanut.

Mahrous et al. (2015) found that application of ½ NPK + 12 ton and compost + Biofertiizer treatment was more pronounced than other fertilizer treatments as well as the control (NPK). Nitrogen applied at 60 kg fed^{-1} produced the maximum values of growth parameters (Awadalla and Abbas, 2017). Also, they recorded the synergistic effect of Bio-fertilizer treatment on all studied characters in both seasons. In harmony, peanut grown on sandy soil at Ismailia, Egypt, positivelyand differentially responded to application of different types of organic fertilizers, i.e. farmyard manure, solid plant compost, enriched compost tea and/or bio-fertilizer mixtures of *Azospirillum braselinse+ Bacillus megatherium+ Azotobacter* *chroococcum* and *Bradyrhizobium* sp. USDA 3456+ *Serratia marcescens* MH6+ *Psuedomonas fluorescens* IFO 2034, reflecting different seed yield and shelling percentage (Elbaalawy et al. 2020). Superiority in yield and yield components from treating seeds of peanut by Rhizobacterin inoculation may be attributed to N_2-fixation, which had marked influence on the growth of peanut plants and reflects to increase yield and yield components.

Nitrogen uptake (kg ha^{-1})

Nitrogen uptake by roots tended to increase with organic additives comparing to the non-treated plants (Fig. 2). This was true with either inoculated or un-inoculated plants. It seems that application of leuceana residues have a potential to increase N uptake by roots over the animal manure and quail feces, respectively. This increase in N uptake was more vigorous under bacterial inoculation rather than the un-inoculation treatment. Comparatively, leuceana residues resulted in relative increase in N uptake by about 18%, 24%; 24%, 101% over animal manure, quail feces for un-inoculated and inoculated plants, respectively. This phenomenon was confirmed by the mean average that clarified the superiority of leuceana residues over others including the untreated control and in the same time, N uptake by roots of inoculated plants overcome those of the un-inoculated one. Despite of organic additives, the mean average of inoculation reflected a relative increase in N uptake by roots of inoculated plants accounted for 21% over the un-inoculated ones.

Higher amounts of nitrogen uptake were detected in seeds and straw while the lowest N uptake recorded with seed shell. In most of organic treatments, inoculated plants taken up more nitrogen than the un-inoculated plants. On overall means basis of N uptake by seeds, comparison between organic additives indicated the superiority of animal manure over both of leuceana residues and quail feces. All organic additives has enhanced N uptake by seeds and straw comparing to the un-treated control. Laxminarayana (2004) stated that integrated application of organic manure plus inorganic fertilizer induced higher uptake of N, P and K by groundnut compared to that of sole organic manures application due to the increased nutrients availability. In this regard, Hellal et al. (2014) attributed the enhancement of nutrient availability to more vegetative growth and root growth, which release hydrogen ions, phenolic compounds and organic acids as well as acidification effect of manure applied that helped in increasing nutrients availability and uptake of N, P and K by faba bean plants. In accordance, Tiwari et al. (2002) and Celik et al. (2004), have also reported that the inclusion of manure in the fertilization schedule improved the organic carbon status and available N, P, K and S in soil, sustaining soil health and soil physical properties. Concerning the effect of bacterial inoculation, the treatment of *Bradyrhizobium* spp. strains combined with phosphorus fertilizer induced the best nitrogen accumulation rates in different groundnut parts (Didagbé et al. 2014). Similarly, Argaw (2017) found that the highest total biomass yield, kernel yield and plant N accumulation of peanut were found where *Bradyrhizobium* in conjunction with organic fertilizer was applied. This shows the importance of organic fertilizer application in increasing 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 the organic inputs at a late stage of the plants. It has been known that organic fertilizer is the major source of mineral nutrients (Eghball et al. 2002). In addition, organic manures, i.e. farm yard manure and water hyacinth compost either applied individually or in combination with *Rhizobium* inoculation significantly increased both shoot N and P percentages (Sulfab et al. 2011). The synergistic effect of recycled manure could be attributed to the improvement of soil physical structure and water conditions, thereby promote crop growth and nutrients acquisition (Celestina et al. 2019).

On the other hand, with the mineralization and decomposition of organic matter, partial nutrients including P will be released for crop utilization. Several studies also indicated that the conversion of non-soluble P to soluble-P could be achieved in the case of manure application (Zafar et al. 2017; Andriamananjara et al. 2018).Macro and micronutrients (N, P, K, Fe and Zn) uptake by chickpea grainswere increased with application of combined bio-fertilizer and biogas slurry in pot trial (Akhtar et al. 2017). The rhizospheric microbial population also required these nutrients and their inoculation resulted in enhanced colonization of rhizobacteria that make available essential nutrients by solubilization and mineralization resulting in higher uptake by the plant and thus higheraccumulation. The inoculation of Rhizobium increased the nodulation that resulted in increased supply of nitrogen to plant and rhizospheric bacteria, and also increased their activity in the rhizosphere (Ahmad et al. 2011; 2013).

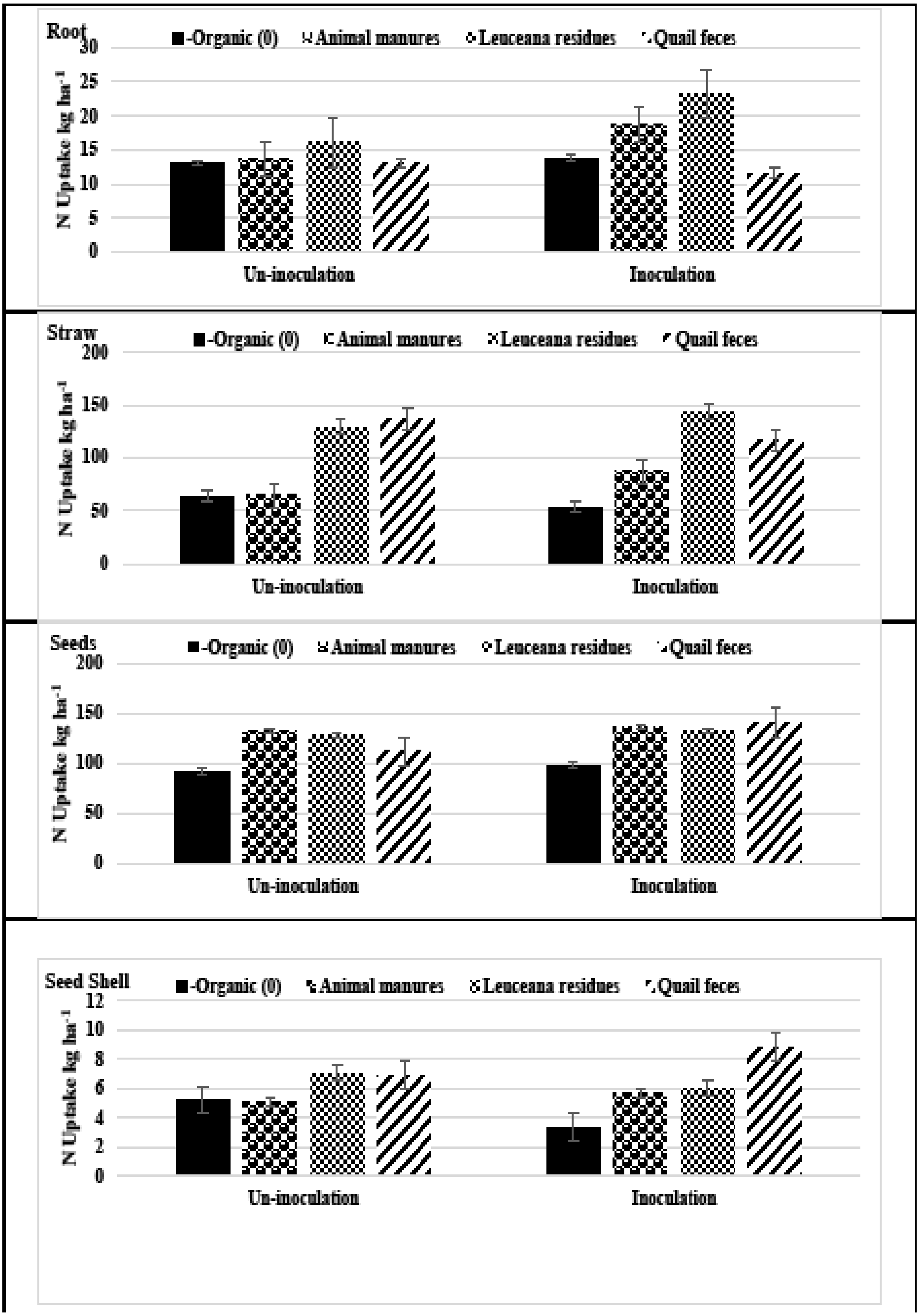

Fig. 2. Effect of Rhizobium inoculation and organic additives on N uptake by roots, straw, seed shell and seed yield (kg ha^{-1}) of peanut crop grown on sandy soil

$^{14}N/^{15}N$ ratio analysis

Nitrogen derived from fertilizer – Ndff, N-remained in soil and N-losses

This portion of nitrogen gained by different peanut organs either as percent or absolute value, in general, tended to decrease in the presence of organic additives comparing to the un-treated treatment (- organic) (Table 3). In the same time, it was higher in case of the un-inoculated plants than the inoculated one. Based on the mean averages, more fertilizer-N was gained by seeds over those gained by straw and roots, respectively. On the other hand, very low amount of fertilizer-N was estimated in seed cover. Absolute values of fertilizer-N gained by root was higher, on mean average base, in case of leuceana residues than those of animal manure and quail feces, respectively. In this regard, it relatively increased by about 12% and 56% over animal manure and quail feces, respectively. Similar trend, but to higher extent, was noticed with Ndff by straw and seeds. Fertilizer-N derived by seed shelldidn't reflect significant variations between organic additives but all of them were lower than those of un-treated control.Concerning the effect of bacterial inoculation, data indicated that Ndff as absolute values based on mean average was relatively varied between negative and positive percentages by about -24%, -42%, +32% and -42% for root, straw, seeds and seed shell, respectively.

Recently, Junior et al. (2020), reported absolute values of Ndff by root, shoot and grains of soybean plants closed to some extent with those obtained in our study. They added that Ndff shoot, Ndff root and Ndff plant were influenced by the rate of N applied where it increased linearly with increasing N rate, independent of the environment or date of fertilizer application.

Portion and absolute values of fertilizer-N fraction remained in soil after harvest was to some extent higher under the un-inoculated condition than inoculated one. These pattern was seems to be lower in case of leuceana treatment than those of either animal manure or quail feces in addition to the un-treated control. Generally, this portion of fertilizer-N kept in soil after harvest was very low. In this respect, under conditions of un-treated (- organic) control, about 2.22 kg N ha^{-1} of added fertilizer to the un-inoculated plants was lost from media by any mechanism taken into consideration the sum of fertilizer-N taken by different organs. While, under the inoculated plants conditions this amount of fertilizer-N was accounted for 1.19 kg N ha^{-1} which means that bacterial inoculation could be accepted for help in minimizing fertilizer-N losses from soil.In a field experiment conduct under tropical and subtropical conditions of Brazil, the N-recovered in soil after soybean harvest was ranged from 22% up to 56% depending on environment and date of fertilizer application (Junior et al. 2020). In comparison, it seems that our results in this respect were very low and explain that organic additives and microbial inoculation contribute in minimizing the portion of fertilizer-N remained in soil after harvest. The losses of fertilizer-N tended to increase with application of organic sources. It means that plants seems to be more dependent on organic and fixed nitrogen rather than mineral fraction. In this regard, losses were accounted for 42.42, 33.65 and 33.33 kg ha^{-1} under the un-inoculated plants treated with animal manure, leuceana residues and quail feces, respectively. In case of inoculated plants, these values were 44.17, 38.91 and 39.46 kg ha^{-1} for the same sequence. It is obvious that losses were increased under inoculation comparing to the un-inoculated one. This also, confirmed the dependence on fixed-N derived to inoculated plants from air rather than mineral form derived from fertilizer.

Nitrogen derived from air – Ndfa

Nitrogen fixed by different plant organs was ranged from 60% up to 75% depending on organic additives (Table 4). Root of un-treated (- organic) plants gained more than 70% of its nitrogen from air recording 9.77 kg ha^{-1} while it accounts for 40.22 kg ha^{-1}, 96.9 kg ha^{-1} and 2.31 kg ha^{-1} in straw, seeds and seed shell, respectively. These values in different parts tended to increase with application of animal manure where it ranged from 4.25 kg ha^{-1} in seed cover to 63.03 kg ha^{-1} in seeds. Relatively, Ndfa by roots, straw, seeds and seed shell of animal manure treated plants was increased by about 39%, 57%, 90% and 84%, respectively over the un-treated control. Similar trend was noticed with leuceana residues but with higher extent, especially in case of root and straw, than those of animal manure treatment.High Ndfa values were derived by seeds and straw, respectively of plants treated with quail feces recording 100 and 67.34 kg ha^{-1}, respectively followed by those of roots (6.92 kg ha^{-1}) and seed shell (6.63 kg ha^{-1}).

TABLE 3. Nitrogen derived from fertilizer (%Ndff, kg ha-1) by peanut parts and portion remained in soil after harvest as affected by bacterial inoculation and organic additives

Inoculation (I)	Organic additives (O)								Mean	
	-Organic (0)		Animal manures		Leuceana residues		Quail feces			
	Root									
	%	kg	%	kg	%	kg	%	kg	%	kg
Un-inoculation	46.40	6.10a	7.30	0.99c	6.90	1.11a	7.10	0.92a	16.93	2.28
Inoculation	24.20	3.30c	7.20	1.35a	6.50	1.51a	6.50	0.75a	11.10	1.73
Mean	35.30	4.70	7.25	1.17	6.70	1.31	6.80	0.84	14.01	2.01
	Straw									
Un-inoculation	29.13	18.50a	8.50	5.44a	9.12	11.81a	9.50	13.05a	14.06	12.20
Inoculation	22.40	11.92b	4.20	3.64b	4.54	6.53b	5.14	6.10b	9.07	7.05
Mean	25.76	15.21	6.35	4.54	6.83	9.17	7.32	9.61	11.56	9.62
	Seeds									
Un-inoculation	18.44	24.14b	4.70	4.30a	5.34	6.81a	5.12	5.71ab	8.40	10.24
Inoculation	27.00	36.59a	4.50	4.10a	4.90	6.48a	4.80	6.80a	10.30	13.50
Mean	22.72	30.37	4.60	4.2	5.12	6.65	4.96	6.26	9.35	11.87
	Seeds shell									
Un-inoculation	35.40	1.84a	2.94	0.15a	3.16	0.22a	2.74	0.19a	11.06	0.60
Inoculation	27.10	0.90b	2.52	0.14a	2.90	0.17a	2.22	0.19a	8.69	0.35
Mean	31.25	1.37	2.73	0.14	3.03	0.20	2.48	0.19	9.87	0.46
	Fertilizer-N remained in soil									
Un-inoculation	2.3	1.2a	1.3	0.7a	0.7	0.4a	1.5	0.8a	1.5	0.8
Inoculation	0.2	0.1d	1.2	0.6a	0.8	0.4a	1.3	0.7a	0.9	0.5
Mean	1.3	0.5	1.2	0.6	0.7	0.4	1.4	0.7	1.2	0.6

Values in the same column followed by the same letter are not significantly different at $P \leq 0.05$.

TABLE4. Nitrogen derived from air (%Ndfa, kg ha-1) by peanut organs as affected by bacterial inoculation and organic additives

Organic additives	Plant organs								Mean	
	Root		Straw		Seeds		Seeds shell			
	Nitrogen derived from air (Ndfa)									
	%	kg	%	kg	%	kg	%	kg	%	kg
-Organic (0)	70.8	9.77bc	75.6	40.22c	52.0	50.75cd	69.9	2.31c	67.1	25.76
*AM	72.5	13.55b	72.7	63.03b	71.2	96.48a	75.9	4.25b	68.3	32.89
†LR	70.4	16.33a	65.8	94.68a	63.1	83.42b	66.5	3.99b	66.5	49.61
‡QF	60.2	6.92c	57.8	67.34b	70.9	100.0a	75.3	6.63a	66.1	45.22
Mean	68.5	11.64	67.9	66.32	64.3	82.66	71.9	4.29	68.2	41.23

Values in the same column followed by the same letter are not significantly different at $P \leq 0.05$.

*AM, animal manure; † Leuceana residue; ‡ QF, quail feces

The overall means of Ndfa values, despite of organic amendments, reflected higher content in seeds and straw followed by those in roots and seed shell, respectively. Whereas, Ndfa by different plant parts on the basis of overall means indicated higher records accounted for 45.22 kg ha^{-1} with treatment leuceana residues followed by quail feces (45.22 kg ha^{-1}) and animal manure (32.89 kg ha^{-1}), respectively but all of them were superior over the un-treated treatment. In this respect, leuceana additive achieved relative increase in Ndfa values by about 92%, 51% and 10% over un-treated control, animal manure and quail feces, respectively. In conclusion, more nitrogen was gained by seeds from air comparing to other plant parts while application of leuceana residues contribute to enhance the portion of nitrogen derived from air in comparison to other additives.

Ning et al. (2020) indicated that soybean treated with recycled manure which had a synergistic effect was more dependent on N-bio-fixation of legumes rather than mineral fertilizers. This phenomenon was early confirmed by Jagadamma et al. (2008).

Nitrogen derived from organic additives - Ndforg

Portion of N derived from organic additives was varied according to type of additives and bacterial inoculation based on comparison with un-treated, un-inoculated controls (Table 5). Roots derived about 20% - 34% of its nitrogen from organic sources. The un-inoculated plants derived 3.29, 4.05 and 4.52 kg ha^{-1} by roots from animal manure, leuceana residues and quail feces, respectively while it was 3.79, 5.36 and 3.83 kg ha^{-1} by inoculated plants for the same sequence. Overall means of Ndforg by roots was relatively higher in case of leuceana residues by about 33% and 13% over animal manure and quail feces, respectively. Similarly, bacterial inoculation, despite of organic sources, induced relative increase in Ndforg by roots accounted for 10% over the un-inoculated plants.

TABLE 5. Nitrogen derived from organic sources (%Ndforg, kg ha^{-1}) by peanut parts as affected by bacterial inoculation and organic additives

Inoculation (I)	Organic additives (O)						Mean	
	Animal manures		Leuceana residues		Quail feces			
				Root				
	%	kg	%	kg	%	kg	%	kg
Un-inoculation(I_0)	24.2	3.29a	25.2	4.05b	34.8	4.52a	28.1	3.95
Inoculation (I_1)	20.3	3.79a	23.1	5.36a	33.3	3.83a	25.6	4.33
Mean	22.3	3.54	24.2	4.71	34.1	4.17	26.8	4.14
				Straw				
Un-inoculation (I_0)	25.2	16.13b	21.6	27.97c	30.8	42.32a	25.9	28.81
Inoculation (I_1)	21.1	18.29a	29.7	42.73a	37.1	43.22a	29.3	34.75
Mean	23.1	17.21	25.6	35.35	33.9	42.77	27.6	31.78
				Seeds				
Un-inoculation (I_0)	45.2	41.18a	39.1	49.70a	29.6	32.94a	37.9	41.27
Inoculation (I_1)	43.5	42.46a	32.0	42.30b	22.3	31.46a	32.6	38.74
Mean	44.4	41.82	35.6	46.0	25.9	32.20	35.3	40.01
				Seeds shell				
Un-inoculation (I_0)	21.6	1.08a	21.1	1.48a	22.2	1.53b	21.6	1.36
Inoculation (I_1)	20.6	1.15a	29.3	1.76a	21.8	1.92a	23.9	1.61
Mean	21.1	1.11	25.2	1.62	22.0	1.72	22.8	1.48

Values in the same column followed by the same letter are not significantly different at $P \leq 0.05$.

In case of straw, this portion was increased as compared to roots. Likewise, Ndforg by straw has the same pattern of roots. In this regard, Ndforg by straw was higher from quail feces than leuceana residues and animal manure, respectively. Relatively, it increased, on mean average basis, by about 21% and 48% over leuceana residues and animal manure, respectively. Also, inoculation resulted in relative increase accounted for 35% over the un-inoculated one. Higher Ndforg by seeds from leuceana residues than from animal manure and quail feces, respectively. Values were slightly higher than those recorded with straw but significantly higher than those derived by roots. In conclusion, portion of N derived from organic sources was higher in seeds and straw than roots and seed shell, respectively. Differentiation between the different organic sources was varied according to plant organ. Also, bacterial inoculation reflected an enhancement effect on increasing the availability of N from the different organic sources.

Conclusion

Recently, more attention has been paid to the contamination of surrounding environment due to excessive use of agrochemicals in agricultural systems. Therefore, turn to organic and bio-agriculture, with special cautions, was put into consideration. Results of the present study confirm that application of organic additives has a positive and significant effects on production and nutritional value of groundnut crop. Organic sources were differentiated among themselves in increasing crop production and nitrogen content in different organs. This may be attributed to their ability in releasing nutrient to plant and also its contribution in improving soil conditions. In general, leuceana residues was more effective in releasing organic-N to crop (Ndforg) and in the same time reducing the portion of N derived from mineral fertilizer (Ndff), minimizing N remained into soil after harvest and consequently reduced N losses from soil media. Bradyrhizobium inoculation compensated considerable amounts of nitrogen via biological N_2-fixation. These values were enhanced in the presence of organic additives which may attributed to the role of released substances from organic materials that affects the activity of bacteria in fixation of N_2 from air. It was proved that groundnut crop was more dependent on N derived from air and those derived from organic sources came to the next. Our results reflected promising perspectives for achieving remarkable yield with minimizing environmental risks and production costs. In the same time, this prospects should be taken with precautions.

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This article does not contain any studies with human participants or animals performed by any of the authors.

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Author contribution

All authors of this study shared in all stages from the beginning with idea, design and experimental work up to interpretation of data and edit of manuscript for publication.

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