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Bradyrhizobium and humic substances fertigation improved fertility and productivity of drip-irrigated sandy soil: Field observations on peanut (*Arachis hypogaea* L.)

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ABSTRACT

Two field experiments were carried out during 2016 and 2017 summer seasons in Randomized Complete Block Design (RCBD) with five replicates under the ecological conditions of Noubaria District, Beheira Governorate, Egypt to evaluate the applicability potential of *Bradyrhizobium* and humic substances fertigation for improving soil fertility and productivity of peanut grown on a drip-irrigated sandy soil compared to compost and mineral fertilizers applications. Physicochemical analysis revealed the high nutrients content of humic substances as well as their nano-sized diameter and abundance of active functional groups. Organic inputs (compost in particular) improved water retention and the lateral water movement in the rhizosphere compared to the prevalence of gravity-dominated vertical movement in the mineral fertigation treatment. Compost application recorded the highest soil organic matter content, which decreased soil pH value. However, *Bradyrhizobium* and humic substances fertigation exhibited the highest concentrations of available nutrients (N, P and K) and nodulation parameters (nodules number and their dry weight) compared to compost and mineral fertilizers. Although mineral fertigation induced the highest vegetative growth characters, *Bradyrhizobium* and humic substances fertigation elicited the highest quantitative and qualitative yield indices. The current study, provides insights into the potential fertigation of N-fixing bacteria alongside with humic substances for maximizing water and nutrient supply potentials of drip-irrigated sandy soils.

Keywords: Peanut, Humic substances, *Bradyrhizobium*, NPK fertilization, Drip irrigation, Sandy soils.

Introduction

Growing crops in sandy soil is vulnerable to intensive synthetic inputs. As a result, understanding the efficient strategies for improving agricultural production in these soils has recently received much attention. Ecological intensification is an innovative strategy that promote water and nutrient supply potentials of soil with the minimum use of synthetic inputs (Tilman *et al*., 2011). Organic farming system has been a typical framework for ecological intensification. Such system has many environmental and nutritional benefits; however, its low yield compared to conventional farming methods has been a major challenge. For instance, crop yields in organic farming system in a 21-year long study were lower than those of conventional mineral nutrition by about 20% (Mäder *et al*., 2002). Another long term experiment carried out in China between 1980 and 2010 suggested that mineral fertilizers could be partially replaced by organic manures to sustain the high maize yield until the soil organic carbon reaches the threshold of $\sim$ 42 Mg C ha^{-1} (Hui *et al*., 2017).

The most common technique for rebuilding soil organic carbon of sandy soil depends on incorporating organic manures (compost in particular) into the plow layer (the rhizosphere) in order to maximize their water and nutrient supply potentials. However, huge amounts of these additives are applied to ensure a significant impact with extra costs of manpower. Further to this, organic manures can be a source of pathogens and weed seeds (Ingham *et al*., 2004). On the other hand, composting inherently contributes in increasing greenhouse gases (GHGs) emission including CO_2, CH_4 and N_2O

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(Sánchez *et al*., 2015). For instance, GHGs emission during composting is estimated as 789,000 Mg of CO_2 eq., representing 2.1% of the total agricultural activities contribution and 0.18% of the *California's annual statewide* GHGs *emission* (Zhu-Barker *et al.,* 2017).

Within the context of the concept "ecological intensification", eco-friendly natural products (e.g. humic substances and biofertilizers) can provide several stimulating mechanisms for promoting plant productivity under different stress conditions. Legumes crops can be considered as typical crops for ecological intensification in sandy soils based on their low water and nutrient requirements and their significant contribution in symbiotic N_2 fixation. According to Kermah *et al.* (2018), annual N_2 fixation by legumes in poorly fertile soils can reach up to 123 kg N ha^{-1}. Peanut (*Arachis hypogaea* L.) is a typical leguminous crop for ecological intensification in sandy soils. However, the low retention of water and nutrients in sandy soil matrix causes a significant reduction in crop productivity due to the large amount of fruitless flowers (Zhao *et al*., 2015). Peanut is considering one of the most important crops success in the newly reclaimed sandy soils as a leguminous crop; contain high nutritive value and an essential source of edible oil and is the fifth important oil seed in the world come after soybean, cotton, canola and sunflower. Peanut seed contains about 25- 30% digestible protein, 45 - 50% oil, 20% carbohydrate and 5% fiber and ash, which make a vital role to human nutrition, Ahmad and Rahim (2007). In Egypt, the total cultivated area of peanut reached about 62000 ha and the total production exceeded 199000 tonnes. However, the total cultivated area in the world reached about 27.940 million ha produce about 47.097 million tonnes, FAO (2019).

The symbiotic interactions between leguminous crops and N-fixing bacteria (rhizobia) involved a molecular "crosstalk" between the two partners in order to invade the plant roots and trigger the formation of nodules. Plant roots release flavonoids, a group of aromatic compounds generated from the secondary metabolism of plant, into the rhizosphere as the first signal for the molecular crosstalk between leguminous crops and rhizobia. These compounds activate the bacterial transcriptional regulatory protein NodD, which is responsible for inducing the translation of other rhizobial nodulation genes (*nod, nol* and *noe*) involved in the synthesis and secretion of the main bacterial nodulation signals (Spaink 2000). Recently, it has been reported that water soluble humic substances contain flavonoid analogue, which might enhance expression of *nod* genes related to N_2 fixation (Gao *et al.,* 2015).

Humic substances, the final component of organic matter decomposition, can be considered as the optimum amendment for sandy soil reclamation given its recalcitrant nature that can support carbon sink in soil. Following its soil application, humic substances tend to improve water and nutrients retention in the root zone, thereby improving water and fertilizers use efficiency (Mosa 2012). The bio-stimulation of plant growth by humic substances is associated with several motivating mechanisms to the primary and secondary metabolisms involved in stress alleviation (Canellas *et al.,* 2015). Besides, Humic substances stimulate enzymes linked to nitrogen assimilation pathways (nitrate reductase, glutamate dehydrogenase and glutamine synthetase) and thus promote nitrogen metabolism in plant (Hernandez *et al.,* 2015, Zanin *et al.,* 2018). Humic substances also encourage photosynthesis (particularly under stress conditions e.g. drought) *via* increasing the rate of gas exchange and electron transport flux in plants (Lotfi *et al.,* 2018). In addition, humic substances stimulate secondary metabolism and thus improve the induced resistance of plant against stress conditions. For instance, humic substances showed a beneficial effect against drought stress through maintaining water absorption and cell turgor/cellular swelling (Khorasaninejad *et al.,* 2018). Additionally, humic substances can regulate the overproduction of reactive oxygen species (ROS) generated under stress conditions through: (i) activating the antioxidative enzymatic system through interaction with the plant radicular system, and (ii) stimulating the synthesis of non-enzymatic compounds linked to shikimic pathway (phenols, alkaloids and tocopherols), which regulate ROS formation by the abundance of reversible redox sites that act as electron carriers (El-Banna *et al.,* 2018).

The combined application of humic substances and rhizobial inoculation, therefore, can provide a cost-effective technique for ecological intensification of drip irrigated sandy soils. Various investigations have explored the effectiveness of humic substances application *via* drip irrigation systems (Mosa 2012, Selim and Mosa 2012, Selim *et al.,* 2009). The novelty aspect of this study relies on applying N-fixing bacteria *via* drip irrigation system either with mineral or organic fertilizers. This innovative technique can provide a long-term simultaneous localization of bioactive materials with high potentiality for ecological intensification of drip-irrigated sandy soils. The specific objectives of this study, therefore, are to: (1) study the integrated effect of mineral fertilizers and eco-friendly natural products (compost, humic substances and *Bradyrhizobium*) application on soil water retention and distribution in the rhizohsphere compared to mineral fertigation system, (2) explore the beneficial effect of experimental treatments on nutrient supply potentials of soil, and (3) evaluate the stimulating effect of experimental treatments on nodulation, growth, yield and nutrients uptake by peanut.

Materials and Methods

Experimental site and layout of the experimental treatments

Two field experiment was carried out at a private farm (30° 30 N latitude and 30° 20 E longitude) located at Noubaria District, Beheira Governorate, Egypt during the growing summer seasons of 2016 and 2017. Values of meteorological data as average over both seasons are presented in Table 1. The experimental soil was sandy in texture (*Entisol-Typic Torripsamments*), and soil analyses were carried out according to Jackson (2005) and presented in Table 2. Irrigation water was obtained from groundwater source, and water samples were taken at each irrigation for analyzing electrical conductivity (Ec) and sodium adsorption ratio (SAR) according to Chapman and Pratt (1962). Following FAO guidelines (Ayers and Westcot 1985), water quality was acceptable for irrigation (average values of Ec and SAR were 0.66 dSm^{-1} and 2.75, respectively).

Treatments were distributed in Randomized Complete Block Design (RCBD) with five replicates, five fertigation treatments were applied as follow: T_1 (full recommended dose of mineral NPK), T_2 (half recommended dose of mineral NPK plus humic substances), T_3 (half recommended dose of mineral NPK plus humic substances and *Bradyrhizobium*), T_4 (half recommended dose of mineral NPK plus compost) and T_5 (half recommended dose of mineral NPK plus compost and *Bradyrhizobium*).

Table 1: Values of meteorological data as average over both growing seasons (2016 and 2017).

Meteorological parameters	Air temperature (°C)	Relative humidity (%)	Sunshine hours (h)	Wind speed (km day^{-1})
Values	28.12	67.53	11.25	194.75

Table 2: Some physical and chemical analyses of the experimented soil as average over both growing seasons (2016 and 2017).

Particle size distribution (%)			Water holding capacity	hydraulic conductivity	Organic matter	$CaCO_3$
Sand	**Silt**	**Clay**	**(%)**	**(cm h^{-1})**	**(%)**	**(%)**
80	11	9	21.87	17.3	0.28	4.25

pH	Ec	Available macronutrients (mg kg^{-1})			DTPA – extractable micronutrients (mg kg^{-1})		
	(dSm^{-1})	**KCl– extractable N**	**Olsen's– extractable P**	**ammonium acetate – extractable K**	**Fe**	**Mn**	**Zn**
8.2	0.30	29.1	4.86	95.88	3.10	2.40	0.68

Materials

Compound fertilizers (HI FRETIL, 20–10–5 NPK and 10–3–36 NPK), and humic substances (extracted from composted crop residues using 0.1 M KOH, 1:7 w/v) were purchased from Fertilizers Development Center, El-Delta Fertilizers Plant, Egypt. Peanut *Bradyrhizobia* strain (*Bradyrhizobium japonicum* USDA 3456) was obtained from Agricultural Microbiology Department, National Research Centre, Egypt. Rice straw (chopped into 2-5 cm pieces) was purchased from a private farm to serve as a feedstock for compost preparation. Seeds of peanut (*Arachis hypogaea* L. cv. Giza 6) were obtained from the Agricultural Research Center, Ministry of Agriculture, Egypt.

Compost preparation and analysis

A plastic sheet was placed on soil surface to control leaching from piles during composting process. Chopped rice straw (1000 kg) was mixed with 20 kg of ammonium sulfate and 100 kg of farmyard manure to activate microorganisms. In piles (3.0 m length, 1.0 m width and 0.75 m height), this mixture was moistened with water at 60% water holding capacity and the moisture was considered satisfactory when a hand-full of piles would wet the hand but not drip. Piles were turned for aeration once a week to enhance the aerobic decomposition process, and the composting process was allowed to continue for 105 days. Composite samples were taken from different spots of each pile to carry out the compost quality analyses according to Peters *et al.* (2003): total moisture content using the gravimetric method, bulk density by calculating the mass per unit volume technique, pH and Ec in 1:10 suspension, organic matter using back titration technique (Walkley and Black 1934), and total NPK concentrations after wet digestion using the mixture of sulfuric (H_2SO_4) and perchloric ($HClO_4$) acids (nitrogen using automatic Kjeldahl equipment (Raypa DNP, 2000), phosphorus using the calorimetric determination by spectrophotometer (UV-VIS Auto UV 2602), and potassium using Jenway PFP7 flame photometer). Some physical and chemical analyses of the produced compost as average over both seasons are presented in Table 3.

Table 3: Some physical and chemical analysis of the produced compost as average values as average over both growing seasons (2016 and 2017).

Analysis	Total moisture (%)	Bulk Density (g cm^{-3})	pH	Ec (dSm^{-1})	OM (%)	Total NPK concentrations (%)			C/N ratio	Nematode
						N	P	K		
Values	32.5 ±2.49	0.61 ±0.06	7.1 ±0.43	2.01 ±0.12	47.0 ±3.50	2.1 ±0.12	0.73 ±0.06	0.84 ±0.05	13:1	N.D.

Values are the mean of three measurements ± standard deviation. N.D. means not detected.

Physicochemical characterization of humic substances

Dry matter content of liquid humic substances was determined using the gravimetric method, and contents of both humic and fulvic acids were gravimetrically determined following the certified fractionation method (Kononova 1961). pH value was determined using a pH meter (Jenway 3505 pH/mV/Temperature Meter). Ec value was determined by HANNA (HI9835) Ec meter. Organic carbon/organic matter concentration was determined using back titration method (Walkley and Black, 1934). Cation Exchange Capacity (CEC) of humic substances was determined using the calcium acetate calcium-chloride displacement method at pH 7.0 as ascribed by Sheldrick (1984). Macro- (N, P and K) and micronutrients (Fe, Mn and Zn) concentration was determined after acid digestion with H_2SO_4 and $HClO_4$ mixture (1:1): N using Kjeldahl method, P using the colorimetric method, K by flame photometer and micronutrients using atomic absorption spectrophotometer (Perkin Elmer model 5000).

Surface topographical analysis of humic substances was determined using scanning electron microscope (JEOL JSM-6400), and the surface elemental composition onto humic substances was characterized using energy dispersive X-ray spectroscopy (EDS, Oxford Instruments Link ISIS). The microstructure investigation of humic substances was identified using transmission electron microscopy (TEM, JEOL JEM-2100) following the dispersion of dry sample onto warm deionized water for 20 min and deposition onto carbon coated grid. Surface functional groups onto active surfaces of humic substances were characterized using Fourier Transform Infrared Spectroscopy (FTIR) analysis. Subsample of humic substances was mixed with KBr (spectroscopic grade) at rate of 100:1 (w/w) and compressed into pellets to be analyzed by a Thermo Scientific-Nicolet iS10 within the observable absorption spectra of 4000 and 400 cm^{-1}. Some chemical analyses of humic substance as average over both seasons are presented in Table 4.

Table 4: Chemical analysis of humic substance as average over both growing seasons (2016 and 2017).

Analyses	Humic acid (%)	Fulvic acid (%)	Dry matter (%)	O.M. (%)	C/N ratio	pH
Values	14.8 ±1.25	3.5 ±0.42	24.0 ±1.58	70.0 ±4.43	14.0	7.7 ±0.52

Analyses	Ec (dSm^{-1})	CEC (cmol kg^{-1})	Macronutrients concentration (%)			Micronutrients concentration (mg kg^{-1})		
			N	P	K	Fe	Mn	Zn
Values	0.98 ±0.06	440 ±31	3.90 ±0.31	0.13 ±0.01	3.30 ±0.28	438 ±46	216 ±16	246 ±21

Values are the mean of three measurements ± standard deviation. N.D. means not detected.

Fertigation systems and peanut husbandry

Soil was well prepared using a chisel plough and divided into 10.5 m^2–experimental units (3.0 m breadth and 3.5 m length) with a 1.0 m border strip surrounding each plot. Compost treatments were applied before the last tillage at rate of 10 Mg ha^{-1}, and the soil was left for 15 days before sowing. Under surface drip irrigation system, peanut seeds were sown by hand drilling in ridges (0.6 m spacing) at 0.1 m seed spacing on the 1st of May in both seasons. Drip irrigation lines were twin-wall drip tapes, and the discharge of drippers was 4 L h^{-1} at 1.5 bar working pressure. The recommended dose of NPK fertilization for peanut production under sandy soil conditions (50, 55 and 72 kg ha^{-1} for N, P and K, respectively) was applied from the 3rd week until the 7th week of fertigation program. *Bradyrhizobium* was cultured in a yeast extract mannitol broth medium (Vincent 1970). Cultures were incubated at 28 °C for three days on a rotary shaker until early log phase to ensure population density of 10^9 colony-forming-units. In a sterilized peat moss growth media, *Bradyrhizobium* culture (120 ml of log phase growing culture) was injected to reach 60% of the maximum water holding capacity, mixed gently and left for a week. Cultures of bioinoculants at 500 ml (10^{12} cells ml^{-1}) was diluted to 500 L ha^{-1} and applied through fertigation system at an interval of seven days commencing from 10 days after sowing (DAS) up to 40 DAS (four times). Humic substance was applied at 120 L ha^{-1} at four equal doses in conjunction with the application of bio-fertilizer treatment. All agricultural practices for peanut plants were conducted according to the recommendation of Agricultural Research Center, Ministry of Agriculture except factors under study.

Monitoring of spatial water distribution

Composite soil samples were randomly taken from experimental plots at 0–10, 10–20, and 20–30 cm distances from soil surface and 0–10, 10–20, and 20–30 cm distances from the drippers using an auger after 1 h from terminating irrigations to monitor spatial water distribution in the rhizosphere. Subsamples were taken by hand from composite samples following quartering technique till obtaining the appropriate weight of sample (~5 g) according to ISO 11464 standard. Subsamples were oven-dried at 110°C till weight constant (about 24 h) for the gravimetric determination of soil moisture content. Soil moisture content was determined fortnightly during the fertigation program. Contour maps for spatial water distributions in the rhizosphere were generated to evaluate water supply potential of soil using Surfer Software (Golden Software, Inc., Golden, CO).

Peanut harvesting and chemical analysis

After 9 weeks from sowing, five representative plant samples were picked from the outer ridges of each plot to determine foliage fresh and dry weight $plant^{-1}$, numbers of total nodules $plant^{-1}$ and dry weight of nodules $plant^{-1}$. At maturity stage (120 DAS), all peanut plants in the three inner ridges of each plot were harvested to obtain pods yield (Mg ha^{-1}; Mg=1000 kg) and seeds yield at ~ 12 % moisture content (Mg ha^{-1}). Crop water productivity (kg m^{-3}) was calculated by dividing pods yield (kg ha^{-1}) per quantity of irrigation water applied (m^3 ha^{-1}). Some quality indices of seeds yield (oil and protein concentration) were determined (Association of Official Analytical 1970). Oil concentration was determined by a Soxhlet extractor using the direct gravimetric method of solvent extraction, and protein was calculated by multiplying total Kjeldahl-N by 6.25.

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On the other hand, subsamples (0.2 g) of foliage and seeds were digested in 5 mL of a 1:1 H_2SO_4 and $HClO_4$ acids mixture to determine N, P and K concentrations. Thereafter, NPK uptake was calculated by multiplying nutrients concentration and dry weight yield. In addition, composite soil samples were taken from each experimental plot to evaluate the effect of the experimental treatments on nutrients supply potential and some chemical properties of soil after harvesting.

Statistical analysis

All data were statistically analyzed according Duncan's multiple range test for analysis of variance (ANOVA) at confidence levels of 95% (Gomez and Gomez, 1984) by CoStat (Version 6.303, CoHort, USA, 1998-2004). Standard deviation values were obtained using Microsoft Excel Software (version 2010, Microsoft Corporation, USA). Furthermore, correlation analysis was calculated using SPSS statistical software (17.0 version; SPSS Inc., Chicago, IL) to evaluate the relationship among soil quality indices and pods yield of peanut.

Results and Discussion

Physicochemical characterization of humic substances

SEM micrographs of dry specimens showed shredded sheets in smooth granular surfaces with small pores and cavities appeared at high magnification (Fig.1a).

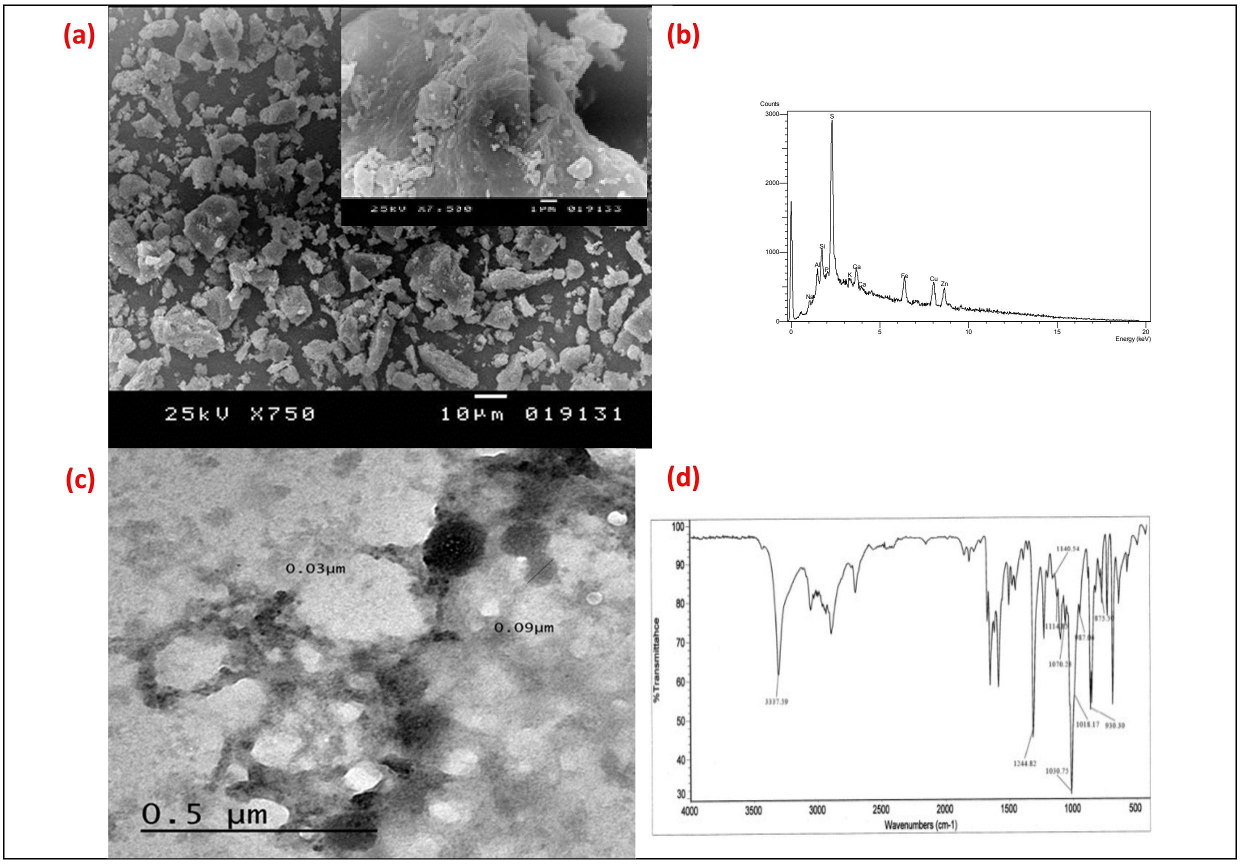

Fig. 1: Spectroscopic analyses of humic substances: (a), SEM photographs at low and high magnifications, (b) TEM micrograph of microstructure features, (c) EDS spectra mapping of elemental distribution onto humic substances surfaces, and (d) FTIR spectra of active functional groups onto humic substances surfaces.

The granular surfaces of humic substances appeared in a compacted phase with a rigid particle structure. The aggregated shape presented in SEM images reflects the cohesion forces between humic substances molecules due to involvement of charge transfer and hydrogen bonding (Das *et al.* 2015). These aggregates appeared in an irregular shape, which confirmed the heterogeneity of humic

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substances. TEM micrographs confirmed also the aggregated shape of humic substances particles, which appeared in a stacked-spherical shape with a nano-sized diameter (Fig.1b). The EDS spectra mapping showed high peaks of Ca, K, Si, S, Al, Cu, Zn, Mg, P and Cl (Fig.1c). The high peaks of these elements reflect the chemical constitution of the feedstock and the extraction solution (KOH).

FTIR spectra of humic substances confirmed the presence of a number of active functional groups: –OH stretching at ∼3337 cm^{-1}; *C*–H stretching at the region between 2900 and 3000 cm^{-1}, C=O stretching and —NH_2 bending at the region between 1650 and 1750 cm^{-1}, C–O stretching at the region between 1244 and 1114 cm^{-1} , S=O stretching at the region between 1030 and 1080 cm^{-1}, and the high peaks located between 700 and 950 cm^{-1} were assigned to C–H bending groups (Fig.1d). Functional groups characterization was identified according to the description of FTIR spectra of former investigations (El-Banna *et al.,* 2018, Mosa *et al.,* 2016, Song *et al.,* 2019, Zhang *et al.,* 2019).

Soil water distribution

Soil water content was uniformly distributed in T_1 compared to other treatments given the approximate parallel distribution of contour lines suggesting a rapid distribution of applied water across soil matrix (Fig. 2). The vertical water movement in the root zone was clearly pronounced in T_1 compared to the lateral movement due to the low water retention in the root zone and the prevalence of gravity force relative to the capillary force in the root zone. In contrary, treatments received organic additives (T_5 in particular) showed an improvement of the water retention and the lateral water movement in the root zone. In this regard, the increase of water content in the root zone of T_2, T_3, T_4 and T_5 was 32.0, 35.4, 37.7 and 40.6%, respectively compared to T_1. Compost application triggered the formation of soil pores (bonding space, residual, and storage pores), thereby enhanced moisture storage potentials in the rhizosphere (Głąb 2014). The pronounced effect of humic substances on improving water retention in the rhizosphere is associated with their physicochemical properties including (i) their porous nature, (ii) their nano-sized diameter and consequently their high specific surface area, and (iii) the abundance of hydrophilic functional groups on their surfaces. On the other hand, there are few reports suggested the positive effect of rhizobia inoculation on soil aggregation around the root system, which improved water retention in the rhizosphere (Alami *et al.,* 2000).

Soil chemical properties

Data of Table 5 showed the effect of experimental treatments on soil pH, organic matter and available concentrations of N, P and K retained in soil after harvesting. Absolute mineral fertigation treatment (T_1) showed a high tendency toward raising soil pH. Meanwhile, the pH value was comparable in other treatments reflecting the improvement of soil buffering capacity following organic additives application. In this regard, pH value was lower in treatments received compost application. Compost has an affinity to reduce soil pH due to generating multiple organic acids during the decomposition process (Muscolo *et al.,* 2018). These organic acids cause an instantaneous reduction in soil pH.

Regarding the effect of fertigation treatments on soil organic matter, it is clearly noticeable that T_5 was the most efficient treatment for increasing soil organic matter without significant difference between T_3 and T_4 in both seasons. Meanwhile, T_1 recorded the lowest soil organic matter content with a reduction of about 24.1% relative to T_5. As mentioned earlier, sole application of organic additives will not be able to sustain the yield intensification in such soil conditions, and supplemental mineral fertilization should be applied until the soil carbon content reaches the threshold (~ 42 Mg C ha^{-1}). Interestingly, it is worth noting that *Bradyrhizobium* inoculation induced an increase in soil organic matter content taking into consideration the values of T_4 and T_5 (0.50 *vs*. 0.52%, respectively as average values over both seasons). This is mainly due to the enrichment of soil microbial community and improving plant roots architecture and nodules formation.

T_3 recorded the highest N, P and K concentrations in most cases. However, T_4 recorded the highest N concentration in second season following the decomposition of soil organic matter and release of available N forms. Beside its key-role as a N-fixing bacteria, there are few reports suggested the potential effect of *Bradyrhizobium* inoculation on solubilizing phosphate precipitates (Abd-Alla 1994). Other reports suggested its role in stimulating the activity of mycorrhiza and phosphorus solubilizing bacteria (Abd-Alla *et al.,* 2001, Omirou *et al.,* 2016). Beside the high concentration of available N in humic substances (3.9 %), the nano-sized fraction and the abundance

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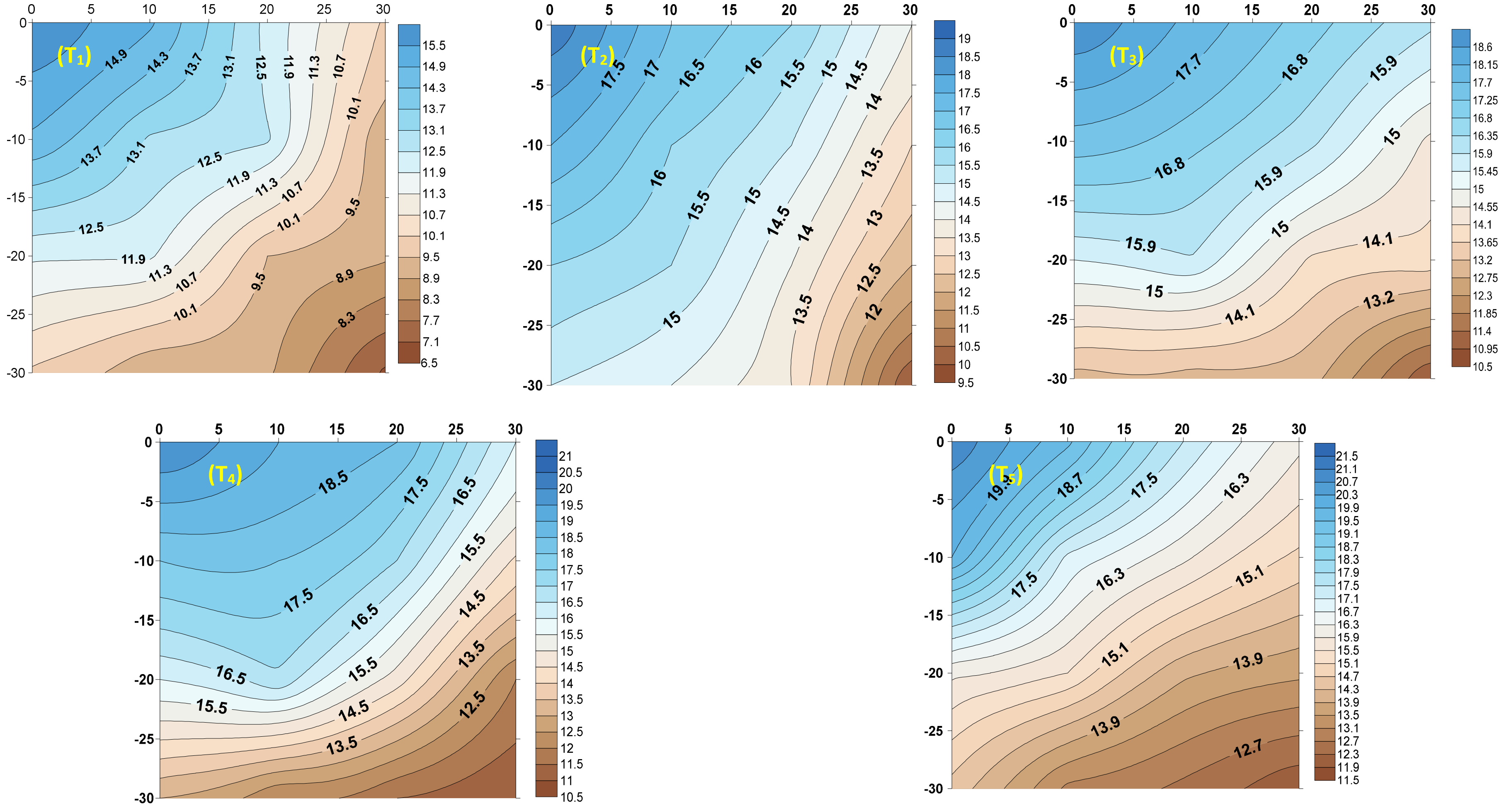

Fig. 2: Average values of spatial distribution of soil moisture content (%) in the rhizosphere (0-30 cm) as affected by fertigation treatments.

Data were obtained as an average values over both seasons**:** T_1 (full recommended dose of mineral NPK), T_2 (half recommended dose of mineral NPK plus humic substances), T_3 (half recommended dose of mineral NPK plus humic substances and *Bradyrhizobium*), T_4 (half recommended dose of mineral NPK plus compost) and T_5 (half recommended dose of mineral NPK plus compost and *Bradyrhizobium*).

of active functional groups onto surfaces of humic substances are able to bind soil available N against losses through leaching and/or volatilization. Humic substances fertigation showed also a significant effect toward maximizing Olsen-P recovered from soil after harvesting. There are several mechanisms responsible for enhancing P availability in soil: (i) the considerable amounts of extractable P in humic substances (0.13%), (ii) complexation of soluble Ca^{2+} ions onto humic substances surfaces allowing phosphate ions to remain available against fixation as calcium phosphate, and (iii) inhibiting the precipitation of hydroxyapatite ($Ca_5(PO_4)_3(OH)$) and favoring the formation of dicalcium phosphate dihydrate (CaH_5O_6P; Delgado *et al.,* 2002). The synergistic effect of humic substances on maximizing K availability is also linked to their abundance of active sorption sites and reactive functional groups against K^+ leaching and the considerable K concentrations derived from organic feedstock and the extracting solution (KOH).

Above- and below-ground biomass

Data presented in Table 6 indicated that T_1 recorded the highest fresh and dry foliage yield in both seasons. This superiority, however, was not significant relative to T_2 and T_3. There is a plenty of research suggested the effectiveness of mineral fertilizers on improving vegetative growth characters and vigour due to the rapid supply of essential plant nutrients (Emami Bistgani *et al.,* 2018, Martínez *et al.,* 2017, Xin *et al.,* 2017). However, substantial alterations in the communities of soil ecosystems and the processes they control are associated with the long-term use of mineral fertilizers due to nutrients leaching and groundwater contamination, eutrophication and substantial alterations in soil acidity. A meta-analysis based on global 107 datasets from 64 long-term experimentation studies illustrated that urea and ammonia fertilizers application caused an inhibitory effect to soil microbial communities due to the temporary increase of soil pH, osmotic potential and ammonia concentrations (Geisseler and Scow 2014).

The high fresh and dry biomass yield of T_3 is associated with the integrated effect between mineral and organic fertilizers application. The partial dose of mineral fertilizers provided a fast-released supply of plant nutrients. On the other hand, humic substances have a biostimulating effect to the pumping activities of H^+-ATPase, which is responsible for ATP hydrolysis leading to excrete H^+ into the apoplast (Canellas *et al.,* 2002). The H^+ production generates a reduction in pH outside the cell and difference in the electrochemical potential across the plasma membrane, which is compensated by cations leading to stimulate plant nutrition. Furthermore, this continuous release of H^+ pumping stimulates the pH-sensitive enzymes and proteins associated with the apoplast leading to cell-wall loosening and growth expansion (Hager 2003). On the other hand, *Bradyrhizobia* strains stimulate phytohormones production: (i) indole-3-acetic acid that accelerates cell division, vascular bundle formation, and nodules formation, (ii) cytokinins that accelerates cell division and root hairs formation, (iii) gibberellins responsible for stem elongation and leaf development, and (iv) abscisic acid, which reinforces plant defense against stress conditions (Gopalakrishnan *et al.,* 2015).

With respect to leguminous crops, optimizing the conditions of soil microbial ecosystem is crucial to activate the nodulation process. Data of Table 6 showed that the absolute mineral fertilization led to dramatic reductions in nodules number and nodules dry weight (8.9 nodules and 376.1 mg plant^{-1} as an average over both seasons). This finding was supported by several reports, which confirmed the inhibitory effect of mineral fertilizers on nodulation activity (Hu *et al.,* 2017, Saturno *et al.,* 2017, Uddin *et al.,* 2008). In the current study, *Bradyrhizobium* inoculation exhibited a vital role in encouraging nodulation process based on the superiority of inoculated treatments (T_3 and T_5). It has been reported that indole-3-acetic acid, a phytohormone that is closely linked to genes responsible for roots nodulation and nitrogen fixation, is produced by *Bradyrhizobium* during the symbiosis process (Liu *et al.,* 2017). On the other hand, nodules number and nodules dry weight showed a positive response to humic substances application. The stimulating effect of humic substances to nodulation activity was obvious in T_3, which induced the superior response (~ 28 nodules and 799 mg plant^{-1} as an average over both seasons). The stimulating effect of humic substances to nodulation activity was recently attributed to several mechanisms including (i) motivating cell density of CCBAU05525, (ii) bacterial activation for better bacteroid development, and (iii) enhancing expression of *nod* gene and N fixation related proteins expression in CCBAU05525 owing to the presence of flavonoid analogue, which might account for its contribution to *nodD1, nodD2, nodA* gene expression (Gao *et al.,* 2015).

Table 5: Effect of different fertigation treatments on some soil chemical properties during the first and second growing seasons (2016 and 2017).

Treatments	pH		Organic matter (%)		Soil available concentration of NPK (mg kg^{-1})					
					N		P		K	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
T_1	8.53^a ±0.10	8.68^a ±0.10	0.36^c ±0.025	0.38^b ±0.019	37.75^b ±0.97	38.30^b ±1.12	9.71 ±0.35	9.06^b ±0.37	110.05^b ±3.06	119.96^{bc} ±3.29
T_2	8.03^b ±0.16	8.17^b ±0.09	0.46^b ±0.018	0.50^a ±0.021	41.07^{ab} ±1.55	40.38^{ab} ±0.92	9.41 ±0.28	9.98^{ab} ±0.49	80.45^d ±2.28	100.86^d ±3.66
T_3	7.99^b ±0.13	7.94^b ±0.10	047^{ab} ±0.015	0.51^a ±0.021	42.13^a ±1.04	38.30^b ±1.18	10.34 ±0.32	11.82^a ±0.22	122.5^a ±3.66	131.7^a ±4.27
T_4	7.93^b ±0.12	7.99^b ±0.10	0.48^{ab} ±0.014	0.52^a ±0.023	37.52^b ±1.06	42.52^a ±0.82	9.85 ±0.37	10.98^a ±0.53	101.4^c ±2.25	115.20^c ±3.43
T_5	7.91^b ±0.15	7.92^b ±0.12	0.52^a ±0.013	0.53^a ±0.016	40.51^{ab} ±0.90	41.64^a ±0.70	9.78 ±0.41	10.34^{ab} ±0.48	116.1^b ±3.57	124.9^{ab} ±4.25

Values are mean of five replicates ± standard deviation. Mean values followed by the same letter are not significantly different at the 5% probability level according to LSD test. T_1 (full recommended dose of mineral NPK), T_2 (half recommended dose of mineral NPK plus humic substances), T_3 (half recommended dose of mineral NPK plus humic substances and *Bradyrhizobium*), T_4 (half recommended dose of mineral NPK plus compost) and T_5 (half recommended dose of mineral NPK plus compost and *Bradyrhizobium*).

Table 6: Effect of different fertigation treatments on above ground biomass and nodulation of peanut during the first and second growing seasons (2016 and 2017).

Treatments	Above-ground biomass				Nodulation			
	Foliage fresh weight (g plant^{-1})		Foliage dry weight (g plant^{-1})		Nodules Number		Nodules dry weight (mg plant^{-1})	
	2016	2017	2016	2017	2016	2017	2016	2017
T_1	79.88^a ±1.12	82.89^a ±1.40	21.71^a ±1.31	25.54^a ±0.81	8.0^c ±0.71	9.8^c ±0.66	311.0^c ± 19.39	441.2^d ± 10.55
T_2	77.29^{ab} ±1.02	79.07^{ab} ±2.04	20.38^a ±0.93	23.85^{ab} ±1.20	20.0^b ±1.00	23.0^b ±1.05	586.0^b ± 37.39	735.36^b ± 9.61
T_3	78.19^{ab} ±0.70	81.63^a ±2.16	21.31^a ±0.87	25.32^a ±1.59	25.2^a ±1.07	31.6^a ±1.21	721.0^a ± 20.27	876.2^a ± 24.88
T_4	73.57^c ±1.01	76.38^b ±1.85	17.15^{ab} ±0.92	20.21^c ±1.17	19.2^b ±0.71	24.6^b ±1.60	559.5^b ± 13.84	666.1^c ± 14.92
T_5	76.08^{bc} ±1.38	78.13^{ab} ±1.82	18.78^{ab} ±0.93	21.78^{bc} ±1.09	21.20^b ±1.32	26.8^b ±1.39	628.5^b ± 24.21	745.2^b ± 17.89

Values are mean of five replicates ± standard deviation. Mean values followed by the same letter are not significantly different at the 5% probability level according to LSD test. T_1 (full recommended dose of mineral NPK), T_2 (half recommended dose of mineral NPK plus humic substances), T_3 (half recommended dose of mineral NPK plus humic substances and *Bradyrhizobium*), T_4 (half recommended dose of mineral NPK plus compost) and T_5 (half recommended dose of mineral NPK plus compost and *Bradyrhizobium*).

Quantitative and qualitative yield indices

Quantitative pods and seeds yield produced by T_3 was higher than other treatments (Table 7). Compared to T_1, the increments of pods and seeds yield of T_3 reached 23.13 and 0.72%, respectively as an average over both seasons. This noticeable disparity in yield increments of pods and seeds is mainly attributed to the noticeable reduction of shelling percentage in T_3 compared with T_1 (55.88% *vs.* 68.07% as an average over both seasons). These results are in great accordance with those obtained by Argaw (2017) who reported that combined application of *Bradyrhizobium* with organic and inorganic fertilizers enhanced the yield indices of peanut grown in Ethiopian sandy soil. The symbiotic performance of *Bradyrhizobium* strains can provide substantial amounts of available N to the leguminous crops. According to Hungria *et al.* (1998), *Bradyrhizobium japonicum* induced accumulation of 84 mg N plant^{-1} in the rhizosphere. The ability *Bradyrhizobium* to provide peanut with its N requirements led to enhance protein concentration in the inoculated treatments (19.02 and 18.48% for T_3 and T_5, respectively). In addition, *Bradyrhizobium* inoculation showed a promoting effect on oil content in seeds, which increased fluidity of membranes and thus cells colonization by bacteria (Silva *et al.* 2013). Another possibility is that *Bradyrhizobium* inoculation promotes quantitative yield characters under drought stress condition through stimulating various metabolic and physiological plant processes. For instance, trehalose biosynthetic genes (otsA, treS, and treY) in *Bradyrhizobium japonicum* are induced under drought stress to provide plant with polysaccharides necessary to sustain the produced yield under such stress conditions (Khan *et al.,* 2016).

On the other hand, the promoting effect of humic substances to yield indices of peanut is mainly attributed to the induction of carbon and nitrogen metabolism. According to Nardi *et al.* (2009), exogenous application of humic substances promoted the activity of enzymes involved in glucose metabolism (glucokinase, phosphoglucose isomerase, aldolase, and pyruvate kinase). Likewise, primary enzymes linked in N assimilation (nitrate reductase, glutamate dehydrogenase, and glutamine synthetase) are also stimulated by humic substances application (Canellas *et al.,* 2015). The stimulating effect of humic substances on oil content in seeds may be attributed to its role in activation of secondary metabolic pathways associated with fatty acids biosynthesis. According to Noroozisharaf and Kaviani (2018), humic acid improved the expression of the phenylalanine ammonialyase, which sparks the biosynthesis of phenylpropanoid through transforming tyrosine to p-coumaric acid and phenylalanine to transcinnamic acid.

Water productivity of T_3 was higher than other treatments over both seasons: T_3 (1.215 kg m^{-3}) > T_1 (0.995 kg m^{-3}) > T_2 (0.855 kg m^{-3}) > T_5 (0.790 kg m^{-3}) > T_4 (0.720 kg m^{-3}). This finding highlights the applicability potentials of *Bradyrhizobium* and humic substances fertigation to maximize water productivity in arid and semi-arid conditions.

Nutrients uptake by foliage and seeds.

N, P and K uptake by foliage and seeds of plants grown in T_1, T_2 and T_3 was comparable with a slight superiority to T_1 (Table 8). Meanwhile, T_4 and T_5 recorded lower values relative to other treatments. Compared to T_1, T_5 recorded reductions in N, P and K uptake by about 26.19, 28.37 and 31.31%, respectively as an average over both seasons. This finding highlights the importance of humic substances to sustain nutrients requirements of drip-irrigated peanut alongside with the partial dose of mineral fertigation. Humic substances induce the activity of H^+-ATPase, and thus stimulate secondary ion transporters and consequently N, P and K uptake (Canellas *et al.,* 2015). For example, the generation of H^+ electrical gradient across the plasma membrane following ATP hydrolysis can provide a motive force for K uptake by plant. Other reports highlighted the promotion of nitrate uptake (the dominant form of soil available N) due to the stimulating effect of humic substances to BnNRT1.1 and BnNRT2.1; genes encoding nitrate transporters (Jannin *et al.,* 2012). A recent study suggested also the stimulating effect of phosphorus uptake due to the key role of phenols and lignin derivatives from humic substances (Spaccini *et al.,* 2019).

Beside the crucial effect of *Bradyrhizobium* inoculation on providing peanut with its N requirements, it seems that it has a stimulating effect on P and K uptake, especially when applied alongside with humic substances. Phytate (the dominant form of identifiable organic phosphorus) is unavailable for uptake by plants. However, this unavailable form can be hydrolyzed by phytase enzyme into readily available form. The abundance of phytase in nodules, and its contribution to the survival of rhizobia–legume symbiosis under deficient-phosphorus soils has been reported (Lazali *et*

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Table 7: Effect of different fertigation treatments on pod and seed yields/ha, shelling %, protein %, oil % and crop water productivity/m^{-3} of peanut during the first and second growing seasons (2016 and 2017).

Treatments	Yield (Mg ha^{-1}) Pod		Yield (Mg ha^{-1}) Seed		Shelling (%)		Protein (%)		Oil (%)		Crop water productivity (kg m^{-3})	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
T_1	3.69^{b} ±0.14	4.48^{b} ±0.15	2.57^{a} ±0.22	2.97^{a} ±0.08	69.57^{a} ±1.61	66.56^{ab} ±1.66	18.30^{ab} ±0.30	19.02^{ab} ±0.47	49.54^{c} ±0.62	49.90^{d} ±0.48	0.90	1.09
T_2	3.31^{bc} ±0.15	3.74^{c} ±0.12	2.27^{ab} ±0.16	2.62^{b} ±0.07	68.84^{ab} ±1.56	70.10^{a} ±1.24	18.84^{a} ±0.14	19.4^{ab} ±0.46	51.39^{b} ±0.48	52.00^{c} ±0.45	0.80	0.91
T_3	4.84^{a} ±0.29	5.22^{a} ±0.14	2.60^{a} ±0.15	2.98^{a} ±0.08	53.74^{c} ±1.62	58.01^{c} ±1.49	19.02^{a} ±0.20	20.36^{a} ±0.44	53.72^{a} ±0.42	53.16^{b} ±0.33	1.18	1.25
T_4	2.81^{c} ±0.13	3.11^{d} ±0.10	1.80^{c} ±0.15	2.17^{c} ±0.11	66.93^{ab} ±1.34	70.47^{a} ±1.18	17.97^{b} ±0.31	18.32^{b} ±0.49	54.47^{a} ±0.30	54.24^{a} ±0.41	0.68	0.76
T_5	3.08^{bc} ±0.24	3.40^{cd} ±0.14	1.92^{bc} ±0.11	2.19^{c} ±0.11	64.04^{b} ±2.06	64.50^{b} ±1.42	18.48^{ab} ±0.21	19.02^{ab} ±0.47	53.34^{a} ±0.49	53.00^{b} ±0.36	0.75	0.83

Values are mean of five replicates ± standard deviation. Mean values followed by the same letter are not significantly different at the 5% probability level according to LSD test. T_1 (full recommended dose of mineral NPK), T_2 (half recommended dose of mineral NPK plus humic substances), T_3 (half recommended dose of mineral NPK plus humic substances and *Bradyrhizobium*), T_4 (half recommended dose of mineral NPK plus compost) and T_5 (half recommended dose of mineral NPK plus compost and *Bradyrhizobium*).

Table 8: Effect of different fertigation treatments on nutrients concentration uptake in foliage and seeds of peanut during the first and second growing seasons (2016 and 2017).

Treatments	Nutrients uptake by foliage (kg ha^{-1}) N		P		K		Nutrients uptake by seeds (kg ha^{-1}) N		P		K	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
T_1	170.24^{a} ±4.61	184.68^{a} ±4.32	69.08^{a} ±2.72	75.46^{a} ±1.94	121.03^{a} ±2.28	146.83^{a} ±4.00	78.30^{a} ±2.94	84.64^{a} ±2.59	8.50^{a} ±0.43	8.94^{a} ±0.23	58.42^{a} ±2.27	71.92^{a} ±1.96
T_2	167.87^{a} ±5.45	183.96^{a} ±4.83	63.05^{a} ±2.33	69.44^{a} ±2.52	112.2^{a} ±3.48	140.11^{a} ±2.75	68.29^{b} ±1.43	72.82^{b} ±3.03	7.78^{a} ±0.26	8.78^{a} ±0.29	49.82^{b} ±1.83	68.02^{a} ±2.08
T_3	163.94^{a} ±5.06	176.50^{a} ±4.21	64.89^{a} ±1.98	70.02^{a} ±1.78	118.03^{a} ±4.45	145.62^{a} ±3.97	76.90^{a} ±2.59	84.04^{a} ±2.54	8.05^{a} ±0.30	8.68^{a} ±0.26	60.22^{a} ±1.65	68.90^{a} ±2.23
T_4	148.16^{b} ±4.30	158.50^{b} ±4.31	53.23^{b} ±2.92	60.22^{b} ±2.32	98.27^{b} ±3.96	115.27^{b} ±3.87	54.14^{c} ±1.63	66.40^{b} ±2.38	5.48^{b} ±0.28	6.48^{b} ±0.16	37.98^{c} ±1.76	44.47^{b} ±2.11
T_5	129.15^{c} ±5.89	132.82^{c} ±4.06	44.08^{c} ±1.99	59.46^{b} ±1.68	85.25^{c} ±2.29	98.75^{c} ±4.71	56.34^{c} ±1.42	66.88^{b} ±2.34	5.74^{b} ±0.31	6.40^{b} ±0.21	45.88^{b} ±2.49	41.38^{b} ±2.46

Values are mean of five replicates ± standard deviation. Mean values followed by the same letter are not significantly different at the 5% probability level according to LSD test. T_1 (full recommended dose of mineral NPK), T_2 (half recommended dose of mineral NPK plus humic substances), T_3 (half recommended dose of mineral NPK plus humic substances and *Bradyrhizobium*), T_4 (half recommended dose of mineral NPK plus compost) and T_5 (half recommended dose of mineral NPK plus compost and *Bradyrhizobium*).

al., 2013). The beneficial effect of *Bradyrhizobium* inoculation was also obvious with potassium in the current study. Such stimulating effect may be associated with the presence of K-uptake related genes in *Bradyrhizobium* genome. According to Domínguez-Ferreras *et al.* (2009), the in silico investigations confirmed the presence of genes responsible for potassium uptake (1021: Kup1, Kup2, Trk, and Kdp) in the genome of *Sinorhizobium meliloti* (a nitrogen fixing bacteria).

Correlation matrix for soil quality indices and peanut pods yield

A Pearson's correlation analysis was performed to analyze the relationships among average values of soil quality indices and peanut pods yield over both seasons (Table 9). It seems that pods yield (Mg ha^{-1}) showed significant correlation ($P < 0.01$) with soil available K ($r = 0.510$) suggesting that this variable is the main limiting factor for pods yield formation. Potassium plays several metabolic functions for peanut production grown in such soil conditions including adjusting water status of plant, regulating stomata functionality, activating photosynthesis and protein synthesis pathways, stimulating N fixation and translocation of photosynthates from leaves into nodules (Almeida *et al.,* 2015). Surprisingly, it is clear that peanut pods yield showed a negative correlation with soil organic matter concentration ($r = -0.311$) suggesting that quality of soil organic matter (humification rate, the presence of active functional groups, aromacity, the nano-sized fraction and nutrients content) is more crucial than its quantity. Soil moisture content showed a high correlation with both of soil organic matter ($r = 0.528$) and soil pH ($r = -0.635$) suggesting the crucial effect of soil moisture content on regulating the decomposition rate of organic matter, thus reducing the generation of organic acids and CO_2. Soil available N showed also a high significant correlation with soil available P (r = 0.550, $P < 0.01$). This finding confirmed the importance of soil available P to the symbiotic fixation of N by *Bradyrhizobium*. Soil available N showed also a significant correlation ($P < 0.05$) with available K ($r = -0.497$) and soil pH ($r = -0.411$). Soil available P showed a negative significant correlation with soil pH (r = -0.511, $P < 0.01$) confirming the vital role of soil pH reduction on phosphate solubilization. Soil organic matter showed a high significant correlation with soil pH (r = -0.806, $P < 0.01$) confirming the aforementioned reduction of soil pH following organic matter decomposition.

Table 9: Correlation matrix for average values of soil quality indices and peanut pods yield as average over both growing seasons.

	Pods yield (Mg ha^{-1})	Soil moisture content (%)	Soil available N (mg kg^{-1})	Soil available P (mg kg^{-1})	Soil available K (mg kg^{-1})	Soil OM (%)	Soil pH
Pods yield (Mg ha^{-1})	1						
Soil moisture content (%)	0.118	1					
Soil available N (mg kg^{-1})	0.374	0.248	1				
Soil available P (mg kg^{-1})	0.251	0.382	0.550**	1			
Soil available K (mg kg^{-1})	0.510**	0.011	0.497*	0.365	1		
Soil OM (%)	-0.311	0.528**	0.358	0.415*	0.024	1	
Soil pH	0.220	-0.635**	-0.411*	-0.511**	-0.098	-0.806**	1

Conclusion

The challenge of maximizing crop productivity of vulnerable soil types (e.g. sandy soils) is to find out ecological intensification strategies for promoting their water and nutrients use efficiency with minimum inputs of synthetic compounds (e.g. mineral fertilizers and pesticides). Symbiotic N_2 fixation using bacterial inoculation has attracted attention since a long time as an eco-friendly technique for crop intensification and intercropping plantation. In this research, *Bradyrhizobium* inoculant and humic substances fertigation was investigated as a modern technique for improving fertility and crop productivity of drip-irrigated sandy soils compared to compost and mineral application treatments. The scientific hypothesis of this research depends on the existence of flavonoid analogue in humic substances that can enhance expression of *nod* genes related to N fixation. *Bradyrhizobium* and humic substances fertigation improved water and nutrient supply potentials of soil and maximized growth and yield of peanut. This technique, can provide a long-term simultaneous localization of bioactive materials with high potentiality for ecological intensification of drip-irrigated sandy soils. However, attempts should be undertaken to improve the viability potential of this technique to compensate the extra cost and labor for procuring humic substances. Further field investigations should be undertaken with several leguminous field crops and symbiotic N-fixing bacterial to establish a holistic approach for widespread application.

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