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INTEGRATED EFFECT OF MINERAL NITROGEN, BIO AND ORGANIC FERTILIZATION ON SOYBEAN PRODUCTIVITY

BY

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ABSTRACT

Partial substitution of mineral nitrogen fertilizers (MNF) through inoculation of soybean seeds with Bradyrhizobium japonicum as a biofertilizer in presence of low dose of mineral nitrogen fertilizer (MNF) (48 kg N ha^{-1}) and complete substitution by using biofertilizer inoculation individually or combined with two different rates of farmyard manure (24 and 48 m3 ha^{-1}) as a N organic source were compared with the recommended dose of MNF (167 kg N ha^{-1}), on soybean growth and yield components have been studied, under field experiment conditions, for two successive summer growing seasons of 2009 and 2010. Obtained results revealed that inoculation of soybean seeds with Bradyrhizobium japonicum, in general, increased nodule numbers, nodules dry weight and nitrogenase enzyme activity as well as microbial population, compared to application of recommended dose of MNF. These increases led to associated increases in N, P and K contents of straw and seed and therefore enhanced yield and yield components of soybean plant. Biofertilizer inoculation + low N dose of MNF, gave the highest values of both nitrogen use efficiency (NUE) i.e. 28.07% and nitrogen uptake efficiency (NPE) i.e. 22.54%. On the other hand, combined treatments of biofertilizer inoculation+48 m^3 FYM ha^{-1}, which represents one of the choice of complete MNF substitution, recorded significant values and best results in both seasons for all the abovementioned parameters associated with soil and plant among the concerned treatments, exception being obtained with NUE and NPE parameters.

INTRODUCTION

Nutrient fertilizers are of growing importance because of the increased demand for higher yielding crops, intensive cropping, and continued expansion of cropping (Bell and Dell, 2006). Such practices exhausted available nutrient and therefore extensive fertilizer applications are required to transform soil from environmental burdens into economic opportunities (Qadir, *et al.*, 2008). However, the pollution accompanied with the heavy use of mineral fertilizer in agriculture concerns an environmental trepidation (Ghosh and Bhat, 1998). For this reason, soil sustainability became of high significance and requires effective management of the soil resources while improving or even maintaining its quality (Bohlool *et al.*, 1992) and this can take place through reducing the inputs of production with increasing their efficiency to obtain high production (ODUM, 1989).

Biological nitrogen fixation BNF is considered an important alternative for N mineral fertilizers (Dobereiner *et al.*, 1995), introduced the large inputs of nitrogen to soil (Bøckman, 1997)and minimize the negative environmental impacts of applying N mineral fertilizers to the soil (Fixen and West, 2002). Successful N_2-fixing bacteria have been found in association with different plants e.g. grass and cereals(Boddey and Dobereiner, 1995) , wheat (Boddey *et al.*, 1986) , rice (Nayak *et al.*, 1986), sugarcane and rice (Boddey *et al.*, 1995), soybean (Zhang *et al.*, 2003) and therefore, soil inoculation with N_2 fixing bacteria is considered an effective way in increasing the nitrogen content in soil (Peoples *et al.*, 1990).

Soybean is one of the most significant crops worldwide (Hartman *et al.*, 2011) and is considered an important source of oil and protein (Keyser and Li, 1992). Soybean oil ranked number one in oil consumption among the major oil seed crops (Singh and Hymowitz, 1999) and represents 54% in the worldwide market (Wilson, 2008). Also, its high protein content in seeds accounts for both feed and food utilization of soybean (Vollmann *et al.*, 2000). High yield production of soybean requires extensive applications of N to soil, and biological nitrogen fixation contributes to provide plants with their N needs at low cost price (Campo *et al.*, 2009), with an average of 50–60% of N demand (Salvagiotti *et al.*, 2008). Calculating N efficiencies for the applied fertilizers is of high importance in this concern as their high values indicate achieving crop demands without excess or deficiency, low cost of production, and low environmental pollution (Cassman *et al.*, 2002; Fageria and Baligar, 2005).

Moreover, some biological N fertilizers e.g. *Bradyrhizobium* excretes indole-3-acetic acid (IAA), gibberellic acid (GA3) and zeatin (Z) in the growth media which increased seed germination, nodule formation, and early development (Cassán *et al.*, 2009). The N_2 fixation process is catalyzed by nitrogenase enzyme system (Kim and Rees, 1994) which decreased with increasing the nitrogen fertilization inputs (Salvagiotti *et al.*, 2008) and with flooding (Sánchez *et al.*, 2011).

During the early stages of soybean growth, depending on N_2 fixation as a sole source for N causes growth retardation as 64% of the photosynthic input of carbon are directed for nodules development (Singleton and van Kessel, 1987) and the amount cannot be compensated by increasing the efficiency of net photosynthesis (Finke *et al.*, 1982); on the other hand, no reductions in yield was reported for the reduced N_2 fixation in early stages of soybean growth (Zablotowicz and Reddy, 2004). Therefore, low nitrogen inputs were used in the early stages of soybean growth to promote nodulation. The amounts of fixed N were found to increase in the following year of application (Peoples *et al.*, 1990).

Amending the soil with farm yard manure (FYM) improves soil physical properties (Haynes and Naidu, 1998) and fertility (Haikel *et al.*, 2000), resulting in an increase in the growth and yield components of different crops i.e. maize (Gajri *et al.*, 1994), rice (Dinesh *et al.*, 1998), wheat (Sushila and Gajendra, 2000), soybean (Hati *et al.*, 2006). Besides, FYM application resulted in an increase in the fungal population (Das and Dakora, 2010), oligonitrophilic bacteria, fungi and actinomycetes counts (Mandic *et al.*, 2011), and microbial biomass carbon (Chauhan *et al.*, 2011)

Intensive efforts are focused on minimizing amounts of applied chemical fertilizers, particularly those of N fertilizers, as well as decreasing the production costs along with reducing the environmental hazards of pollutants. Therefore, the present work was undertaken to investigate the possibility of using bio and organic fertilizers to substitute partially or totally the mineral N ones.

MATERIALS AND METHODS

1. Materials of study

Soils: The soils used in the present work were analyzed according to Page *et al.* (1982) and Klute (1986) and results are shown in Table (1).

Table (1): Physical and chemical properties of the studied soil

Soil characteristics	First season 2009	Second season 2010
Coarse sand (%)	2.29	2.29
Fine sand (%)	10.98	10.48
Silt (%)	29.88	30.7
Clay (%)	56.85	56.33
Textural class	Clayey	Clayey
$CaCO_3$ (g kg^{-1})	25.10	22.31
OM (g kg^{-1})	16.54	14.21
pH	8.26	8.22
EC (dS m^{-1})	1.21	1.13
Available N (mg kg^{-1})	41.00	45.00
Available P (mg kg^{-1})	9.64	8.47
Available K (mg kg^{-1})	398	348

pH: 1:2.5 soil :water suspension; EC: saturation paste extract

Soybean seeds: The seeds of soybean (*Glycine max* L.) cultivar Giza 111were supplied by the Plant Breeding Department, Agriculture Research Center, Giza.

Bacterial inoculums: Rhizobium strains were supplied by Department of Microbiology, SWERI, ARC, Giza. Strains were characterized by effective ability to infect specific host plants and high efficiency in N_2-fixation. Strains were grown on yeast extract mannitol broth medium (Vincent, 1970), mixtures of two strains of *Bradyrhizobium japonicum* USDA 110 and HH303 were added to sterile soil carrier (vermiculite +10% peat) to prepare the inoculant used for soybean inoculation.

Fertilizers used

- **Organic fertilizer :**Farmyard manure (FYM) was applied at three different rates (0, 24 and 48 m^3 ha^{-1}) and some chemical characteristics were analyzed and the results are presented in Table 2

- **Mineral fertilizers:** Mineral nitrogen fertilizer (MNF) was applied at rates of 0, 48 and167 kg N ha^{-1} in the form of ammonium sulphate (20.5% N).

Table (2): Some chemical characteristics of farmyard manure (FYM) used in the field experiment.

Characteristics	Value	Characteristics	Value
pH	7.24	Available N (g kg^{-1})	4.80
EC (dS m^{-1})	4.20	Available P (g kg^{-1})	2.53
Organic matter (%)	40.11	Available K (g kg^{-1})	3.44
Total N (g kg^{-1})	12.63	Bulk density (kg m^{-3})	641.00
C/N ratio	18.42		

*pH and EC of the FYM were measured in 1:10 extract.

2. The field work

A field experiment was conducted at Damas village, Mit Ghamr, Dakahlia Governorate for two successive summer growing seasons of 2009 and 2010 to study the integrated effect of mineral-N, bio and organic manure (fertilization) on soybean productivity. The experiment was laid out according to the randomized complete block design (RCB) with three replicates on a net plot area of 10.5 m^2. Treatments of farmyard manure was applied before soybean planting, and mixed thoroughly with the soil. Soybean seeds were divided into two groups. The first group was sowing at mineral N fertilizer (MNF) at rates of 0 and 167 kg N ha^{-1} to represent control treatment (T_1) and full recommended dose (T_2), respectively. While, the second group was mixed with suitable amount of Arabic gum solution 15 %, as adhesive material, then thoroughly mixed with bacterial inoculants at rate of 10 g /kg soybean seeds. Both groups were cultivated in FYM treatments at rates of 0, 24 and 48 m^3 ha^{-1} (T_3, T_5 and T_6, respectively), that to represent complete substitution of mineral fertilizers, besides the MNF treatment (T4), which introduce the partial substitution treatment. The PK fertilizers were applied to the experimental plots as recommended by the Egyptian Ministry of Agriculture in the form of Calcium super phosphate (15%P_2O_5) and potassium sulfate (48%K_2O) at the rates of 31 kg P $ha^{-1}$and 100 kg K ha^{-1}, respectively. All the agriculture recommended practices were followed as usual including the irrigation processes.

3. Experimental measurements

3.1. Nodulation, estimated enzymes activity and microbial population.

On the 45th and 75th days after planting (DAP), 15 plants from each treatment were removed carefully, washed and nodules were separated The number of nodules per plant were recorded and nitrogenase enzyme activity was assessed in soybean nodules according to the methods described by Leth Bridage *et al.* (1982), then the nodules were oven dried for 78 h at 70°C and the obtained nodules and the microbial population dry weights were recorded.

Soil samples from the rhizosphere area were taken at different periods to evaluate dehydrogenase enzyme activity (DHA) and the microbial population. Where, DHA was determined according to the methods suggested by Casida *et al.* (1964). The serial dilution plate technique was employed to specify the rhizosphere soil actinomycetes, fungi and bacteria as recommended by Johnson and Curl (Johnson and Curl, 1979), followed by isolating actinomycetes, fungi and bacteria using Yeast extract–starch agar medium (Emerson, 1958), Martin's rose Bengal agar medium (Martin, 1950) and nutrient agar medium (APHA, 1992), respectively.

3.2. Growth and yield measurements

Shoot dry weights were obtained at the beginning bloom growth stage. The straw dry weight (defined as all the non seed materials collected at the physiological maturity growth stage of soybean), grain yield, 100-seed weight, number of pods per plant, plant height and number of branches per plant were recorded at the physiological maturity growth stage of soybean.

3.3. Soil and plant analysis

Soil samples were collected from all experimental plots during plant harvesting, air dried and sieved to pass through a 2 mm sieve. Soil pH was determined in 1:2.5 (soil : water suspension) using Beckman pH meter, and available soil N was determined as described by Page *et al.* (1982). The collected plant materials i.e. shoot and seed were oven dried at 70° C for 48h, grounded and sieved in a microwilly mill, then digested by the method described by Peterburgski (1968). Total nitrogen, phosphorus and potassium were determined according to Jackson (1973). The Crude protein was calculated by the following equation:

Crude protein= N% × 6.25, according to Horwitz (1980).

3.4. Data analysis

All data obtained from this study were statistically analyzed using the Minitab 15 statistical software through analysis of variance (ANOVA) and least significant difference (LSD) at 0.05 probability level. The calculations for the different fertilizer N efficiencies (nitrogen use efficiency-nitrogen uptake efficiency-nitrogen harvest index) were considered at the physiological maturity growth stage of soybean.

Nitrogen use efficiency (NUE) was calculated according to Sanford and Mackown (1986) as follows:

$$Nitrogen \cdot use \cdot efficiency \cdot (NUE) = \frac{kg \cdot seed \cdot DW}{kg \cdot N \cdot from \cdot soil \cdot (including \cdot fertilizers)} \times 100$$

Nitrogen uptake efficiency was calculated according to Gallais and Coque (2005) and Valle *et al.*(2011) as follows

$$Nitrogen \cdot uptake \cdot efficiency \cdot (NPE) = \frac{kg \cdot N \cdot uptake \cdot in \cdot above \cdot ground \cdot parts}{kg \cdot N \cdot from \cdot soil \cdot (including \cdot fertilizers)} \times 100$$

Nitrogen harvest index NHI was calculated according to Koutroubas *et al.* (1998) as follows:

$$Nitrogen \cdot harvest \cdot index \cdot (NHI) = \frac{N \cdot uptake \cdot in \cdot seeds}{total \cdot N \cdot content \cdot of \cdot above \cdot groud \cdot parts} \times 100$$

RESULTS AND DISCUSSION

1. Effect of inoculation and nitrogen sources on nodulation and nitrogenase activity

The number of nodules, nodule dry weight and nitrogenase enzyme activity appeared to have reached their maximum values due to the effect of *B. japonicum* inoculation (Fig. 1) compared with un-inoculated treatments (T_1 & T_2). Similar results found that the number and biomass of nodules per plant increased with *B. japonicum* inoculation in *Glycine max* (Zhang *et al.*, 2003). Also, Ibrahim *et al.* (2011) found that soil inoculation with *Bradyrhizobium* resulted in more nodules formation, more uniform distribution of nodules on the roots of soybean, and more nitrogen fixation.

Moreover, data reveal that *B. japonicum* was more effective in soil amended with low MNF dose of 48 kg N ha^{-1}. (T4) than un-amended soil with any dose of MNF (T_1) and these results are in well agreement with those of Tran *et al.* (2007). However, FYM amended soil and *B. japonicum* application (T_5 & T_6) markedly enhanced nodulation and nitrogenase activity comparing with un-inoculated treatments (T_1 & T_2). Conversely, full dose of MNF failed to show effect and suppressed number and biomass of nodules as well as nitrogenase activity. Our results are in agreement with those of Dakora and Phillips (2002) who found that nodulation and N_2 fixation are inhibited by the high N content in soil. After 75 days of planting a pronounced increase in nodulation was observed comparing with that evaluated after 45 days. Nodulation was found to be higher in season 2010 than in 2009 and these results are in well agreement with Shetta (2010) while disagree with Koutroubas *et al.* (1998) who found that the nodules numbers and weights were higher in the first year than the following year. The effect of the different treatments on nodulation of soybean could be arranged as follows: Inoc.+ 48 m^3 FYM ha^{-1}(T_6)> inoc.+ 48 kg N ha^{-1}as MNF (T_4)> inoc.+24 m^3 FYM ha^{-1} (T_5) > inoc. only (T_3).

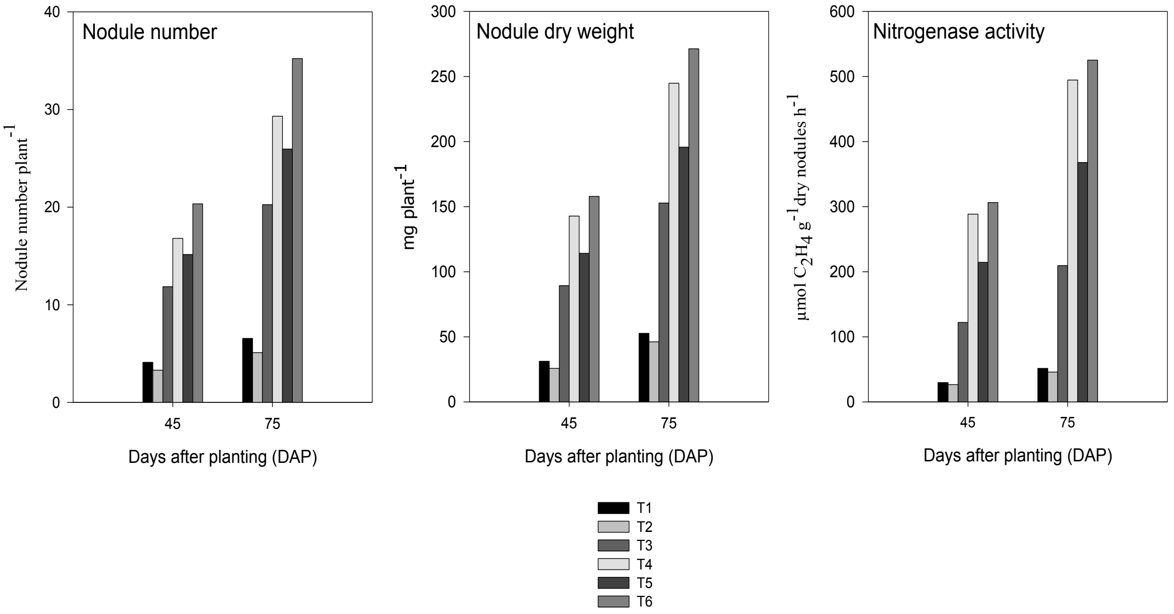

Fig (1): Nodulation and nitrogenase activity of soybean as affected by inoculation and nitrogen sources: uninoculated control treatment (T_1), uninoc.+ 167 kg N ha^{-1} as MNF (T_2), inoc. (T_3), inoc. + 48 kg N ha^{-1} as MNF (T_4), inoc. + 24m^3 FYM ha^{-1} (T_5) and inoc. + 48m^3 FYM ha^{-1} (T_6). Data are mean values of two seasons.

2. Microbial activity in the rhizosphere of soybean plant as affected by inoculation and nitrogen sources

Results in Fig. 2 demonstrate that the microbial population (actinomycetes, fungi and bacteria) and the microbial activity expressed as dehydrogenase enzyme activity in rhizosphere zone of soybean plant was greatly influenced by farmyard manure application along with *B. japonicum* inoculation. The maximum population of the three microbial groups registered among all the treatments were due to applying both the two FYM amendment rates of 24 & 48 m^3 ha^{-1} in the presence of *B. japonicum* inoculation (T_5 & T_6), while the least one was observed in control treatment (T_1), which didn't receive any fertilizers, during the two periods of plant growth (45 & 75 days after planting, DAP) in both growing seasons. Data also reveal that inoculation with *B. japonicum* in combination with low dose of inorganic nitrogen fertilizer (48 kg N ha^{-1} as MNF) resulted in higher microbial population than the use of recommended dose of MNF (167 kg ha^{-1}) only. After 75 days of planting a pronounced increase in microbial population was observed comparing with that evaluated after 45 days.

Moreover, microbial population observed in season 2010 was higher than observed in season 2009. The effect of the different treatments on actinomycetes, fungi and bacteria counts followed the sequence: inoc.+48 m^3 FYM ha^{-1} (T_6) > inoc.+ 24 m^3 FYM ha^{-1} (T_5) > inoc.+ 48 kg N ha^{-1} as MNF (T_4) >167 kg N ha^{-1} as MNF (T_2). Similar results were achieved by Das and Dkhar (2011) who reported that application of organic fertilizers had enhanced the microbial population compared to NPK and control treatments. On the other hand, Chauhan *et al.* (2011) found that the use of inorganic fertilizers resulted in low organic carbon content, microbial counts and microbial biomass carbon of the soil.

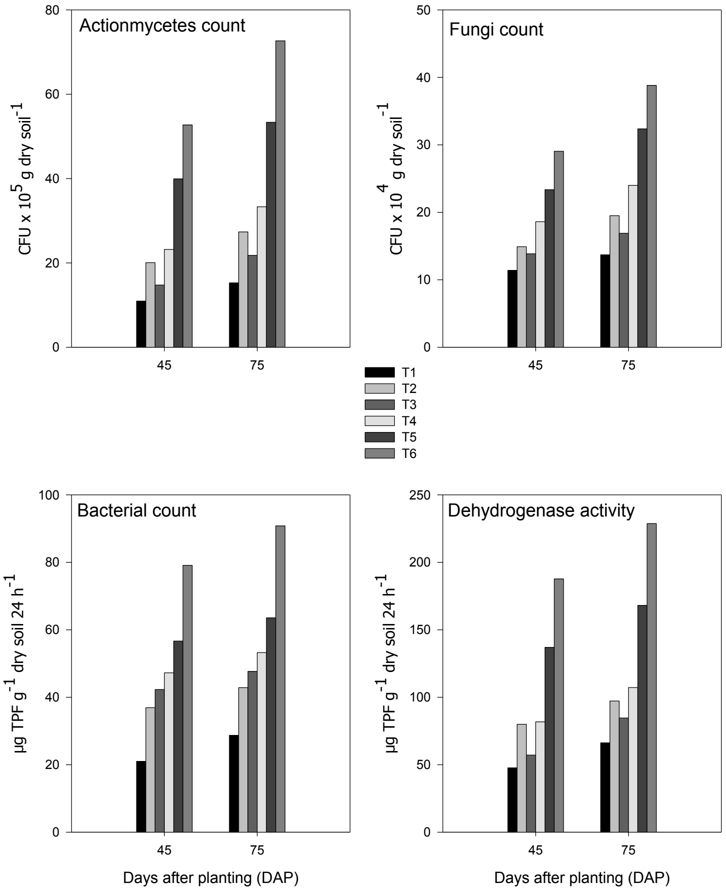

Fig. (2): Actinomycetes, fungal, bacterial counts and Dehydrogenase activity of soybean as affected by inoculation and nitrogen sources: uninoculated control treatment (T_1), uninoc.+167 kg N ha^{-1} as MNF (T_2), inoc. (T_3), inoc+48 kg N ha^{-1} as MNF (T_4), inoc.+ 24m^3 FYM ha^{-1} (T_5) and inoc.+ 48m^3 FYM ha^{-1} (T_6) (Data are the mean values of two seasons).

The organic carbon content of the soil might be enhanced as a result of organic amendment applications and consequently significantly affected bacteria and eukaryotic community structure, resulting in a more diverse and dynamic microbial system than inorganically fertilizer soil as mentioned by Kirchner *et al.* (1993). Our results also are in agreement with Krishnakumar *et al.*, (2005) who found that the microbial population viz., bacteria, fungi and actinomycetes conspicuously increased with application of different organic N sources than the control. The organic manure addition viz., FYM would have resulted in increased micronutrients in the soil which might have helped to increase the microbial population. Moreover, enrichment of soil nitrogen through biological fixation of nitrogen by the host legume plant could have also affected the microbial diversity as mentioned by Bardgett and Shine (1999). Also, Cooper and Warman (1997) found that organic amendments always produced higher dehydrogenase

(DHA) levels than fertilizer amendments.

3. Effect of inoculation and nitrogen sources on nitrogen concentration in seeds and straw of soybean

The results shown in Table 3 reveal that nitrogen content in straw and seeds for plants taken at physiological maturity growth stage increased due to plant inoculated with *B. japonicum* with no N-addition. However, uninoculated plants received 167 kg N ha^{-1} as MNF recorded higher N-content in straw and seeds compared with inoculated plants that received 48 kg N ha^{-1} as MNF. Application of farmyard manure (FYM) had further effect on increasing N content in straw and seeds. The effect of the different treatments on N content in seed followed the sequence: inoc.+ 48m^3 FYM ha^{-1} (T$_6$) >167 kg N ha-1 as MNF (T$_2$) ≈ inoc+48 kg N ha^{-1} as MNF (T4) > inoc.+24m^3 FYM ha^{-1} (T$_5$); however, the N-content in straw followed the arrangement: inoc+48 kg N ha^{-1} as MNF (T$_6$) ≈ inoc.+ 167 kg N kg^{-1} (T$_2$) ≈ inoc.+ 48 kg N ha^{-1} as MNF (T$_4$) > inoc.+ 24 m^3 FYM ha^{-1} (T$_5$). Similar results were found by Koutroubas *et al.* (1998) who found that N content in seeds of soybean increased with *Bradyrhizobium* inoculation. Since it is found that the total nitrogen remained almost constant in soybean plants between seed growth and physiological maturity stages (Koutroubas *et al.*, 1998); therefore, seed fillings depends on the translocation of N compounds from shoots to seeds (Munier-Jolain *et al.*, 1996). Our results show that N-content in shoots at the beginning bloom were much higher than N-content in straw at physiological maturity growth stage, also the order of N-content in straw of soybean obtained at the physiological maturity growth stage due to the different application treatments followed the same sequence of N-content in shoot at the beginning bloom growth stage, and this confirms the redistribution and translocation of N from shoots to seeds during flowering and pod filling.

Table (3): Nitrogen content in shoots and seeds as affected by inoculation and nitrogen sources (Data are the mean values of two seasons)

Treatments	Beginning bloom shoot (mg g^{-1})	Physiological maturity		Seed protein (%)
		Seed (mg g^{-1})	Straw (mg g^{-1})	
T1	42.20e	37.05e	11.00c	23.15e
T2	53.10b	57.75b	18.20a	36.1b
T3	45.30d	44.25d	12.50c	27.65d
T4	52.35b	56.20b	17.30ab	35.1c
T5	49.35c	50.45c	15.85b	35.5c
T6	54.80a	62.60a	19.25a	39.1a

Uninoculated control treatment (T$_1$), uninoc.+167 kg N ha^{-1} as MNF (T$_2$), inoc. (T$_3$), inoc.+48 kg N ha^{-1} as MNF (T$_4$), inoc.+ 24m^3 FYM ha^{-1} (T$_5$) and inoc.+ 48m^3 FYM ha^{-1} (T$_6$).

4. Effect of inoculation and nitrogen sources on phosphorus and potassium concentrations in seeds and straw of soybean

Table 4 reveals that inoculation with *B. japonicum* increased P and K content in shoot at the beginning bloom growth stage and in straw and grain at the physiological maturity growth stage. The application of either FYM or MNF had further increases on P and K contents in shoot and seed at the above mentioned growth stages. These increases could be arranged in the following ascending order: Inoc+48 m^3 FYM ha^{-1} (T_6) >uninoc.+167 kg N ha^{-1} as MNF (T_2) ≈ Inoc.+48 kg N ha^{-1} as MNF (T_4)> Inoc+24 m^3 FYM ha^{-1} (T_5) . Similar results were obtained by Biswas *et al.* (2000) who found that P, and K content increased in rice due to the *B. japonicum* inoculation. Also, Singh and Singh (1993) found that the content of P increased in soybean with *B. japonicum* inoculation. These increases in P and K contents are in well consistent with the increases in the different growth parameters and seed components of soybean and suggests comparable increases in root extensions. These extended roots resulted in more adjacent areas between soil P and K with plant roots, and therefore higher P and K uptake besides the high requirements of the grown plants for P and K. Moreover, the high content of P and K in the FYM treatment, which was applied at high rate of 48 m^3 ha^{-1}, increased their available level in soil and hence their uptake and concentrations in straw and seed of soybean.

Table (4): Phosphorus and potassium concentrations in seeds and straw of soybean as affected by inoculation and nitrogen sources (Data are the mean values of two seasons)

Treatments	Phosphorus (P) (mg g^{-1})			Potassium (K) (mg g^{-1})		
	Beginning bloom shoot	Physiological maturity		Beginning bloom shoot	Physiological maturity	
		Seed	Straw		Seed	Straw
T1	3.43e	2.60f	1.55e	53.00f	12.61f	24.57f
T2	5.78b	4.12b	2.57b	74.52b	19.70b	34.23b
T3	4.79d	2.94e	1.79d	66.20e	14.92e	27.51e
T4	5.73b	3.99c	2.51b	73.55c	18.86c	32.87c
T5	5.43c	3.82d	2.27c	69.86d	16.90d	29.72d
T6	6.02a	4.45a	2.75a	77.11a	20.53a	36.02a

Uninoculated control treatment (T_1), uninoc.+167 kg N ha^{-1} as MNF (T_2), inoc. (T_3), inoc.+ 48 kg N ha^{-1} as MNF (T_4), inoc.+ 24m^3 FYM ha^{-1} (T_5) and inoc. + 48m^3 FYM ha^{-1} (T_6).

5. Effect of inoculation and nitrogen sources on some growth parameters and yield components of soybean

The results shown in Fig. 3 reveal that the studied growth parameters i.e. straw yield, plant height, number of branches, number of pods per plant, 100-seed weight and seed yield were higher in the inoculated plants that didn't receive any N amendment compared with the uninoculated control treatment. Moreover, the applications of either farmyard manure (FYM) or mineral nitrogen fertilizer (MNF) had further increases on these parameters. The increases in the studied parameters due to the treatments could be, generally, arranged as follows: inoc.+ 48 m^3 FYM ha^{-1}(T_6)> inoc.+ 48 kg N ha^{-1}as MNF (T_4) ≈ inoc.+ 167 kg N ha^{-1}as MNF (T_2)> inoc.+24 m^3 FYM ha^{-1} (T_5) > inoc. (T_3)> uninoc. control (T_1).

Similar results were found by other researchers on the enhancement of *B. japonicum* inoculation on the heights and shoot dry weights of *Glycine max* (Zhang *et al.*, 2003). Also, *B. japonicum* inoculation increased 100-seed weight and protein content of soybean seeds (Elsheikh *et al.*, 2009). Moreover, Imsande (1998) found that inoculation with low mineral nitrogen inputs resulted in higher gain yield than the non-inoculated plants fed only on the excess amounts of mineral nitrogen.

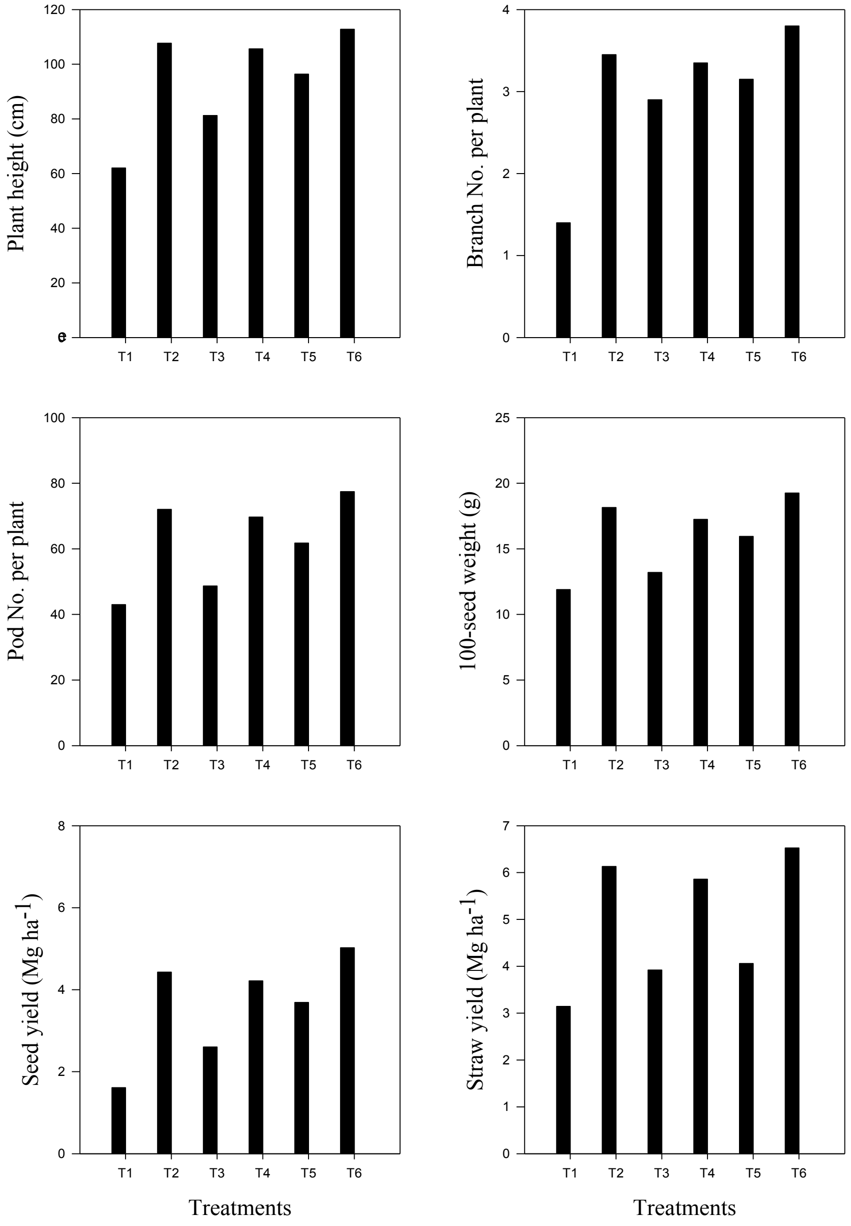

Fig (3): Some growth parameters of soybean as affected by inoculation and nitrogen sources (Data are the mean values of two seasons). Uninoculated control

treatment (T_1), uninoc.+167 kg N ha^{-1} as MNF (T_2), inoc. (T_3), inoc.+48 kg N ha^{-1} as MNF (T_4), inoc. + 24m^3 FYM ha^{-1} (T_5) and inoc. + 48m^3 FYM ha^{-1} (T_6).

Application of FYM to soil enriched soil content with nutritive elements especially micronutrients. The presence of sufficient amounts of micronutrients in soil led to better survival and nodulation for *B. japonicum* (Fouilleux *et al.*, 1996), and thus improved the efficiency of N_2-fixation in soil (Campo *et al.*, 2009), consequently, increased the yield and seed components of soybean (Shetta, 2010).

6. Effect of N availability and its translocation during pod filling on nitrogen content in shoot and seed

Table 5 shows that there are high significant relations between the different growth parameters and yield components with the N-content in straw and seed at physiological maturity growth stage. Likewise, N-content in straw and seed at physiological maturity were significantly related with the N-content in shoot at beginning bloom. The N-content in shoot at beginning bloom was significantly correlated with the initially available N in the studied soil. It is well known that the availability of N in the studied soil is derived from native soil-N, besides N_2 fixed by *B. japonicum* and the organic and mineral N amendments. It was found that the growth and seed yield of the uninoculating soybean plants increased significantly with the increase in the applied N rate in soil (Cure *et al.*, 1988), which affects its availability in soil; besides, the atmospheric N_2 fixation by plant nodules (Berry *et al.*, 2011) which is exported and assimilated in the inoculated plants (Mylona *et al.*, 1995) probable as ammonia rather than ammonium ion (Waters *et al.*, 1998) across the symbiosome membrane (Tyerman *et al.*, 1995).

Table (5): Correlation coefficient values of N-content in shoot and seed, growth parameters and yield components (Data are the mean values of two seasons)

Parameters	Avail-N	N-shoot	N-seed	N-straw
N-shoot (beginning bloom)	0.830*			
N-seed (physiological maturity)	0.813*	0.996*		
N-straw (physiological maturity)	0.834*	0.995*	0.988*	
Straw yield	0.772*	0.656*	0.625*	0.644*
Plant height	0.815*	0.904*	0.912*	0.855*
Branch no. per plant	0.719*	0.867*	0.871*	0.841*
Pod	0.867*	0.911*	0.892*	0.910*
100-seed weight	0.887*	0.904*	0.885*	0.904*
Seed yield	0.833*	0.891*	0.880*	0.883*
Protein content	0.800*	0.974*	0.973*	0.974*

* Significant correlations at the 0.05 probability level.

7. The efficiencies of applied N as affected by inoculation and nitrogen source

The efficiency of applied N is considered an important criteria beside the N- requirements to obtain maximum economic yield (Fageria and Baligar, 2005). Accordingly, the efficiencies of the applied nitrogen for the different bio and organic treatments were calculated and the results were shown in Table 6. These results exhibit that the nitrogen harvest index (NHI) which is the nitrogen content in the seed yield in relation to the total N in the above ground biomass, remained nearly constant except for the treatments no-inoculation control treatment (T_1) and Ino+24m^3 FYM ha^{-1} (T_5). Alves *et al.* (2003) reported no significant effect for either the rate of N applications or *Bradythizobium* inoculation on the NHI values of soybean which ranged from 52-69% . On the other hand, Sanginga *et al.* (1997) found that soybean inoculation decreased the calculated NHI values.

The lowest value of NHI index (62.06 %) was recorded for the uninoculated plants that didn't receive FYM (T_1). This indicates that N translocation from shoot to seed is low and this might be because of the high N requirements of soybean besides the low soil content in N which resulted in the presence of strongly bound N in structural proteins. On the other hand, inoculated plants that received 24 m^3 FYM ha^{-1} (T_5) recorded the highest NHI values, and this indicates high translocation of N from shoot to seed during pod filling.

Concerning the values of nitrogen use efficiency and nitrogen uptake efficiency calculated for the different N- treatments, the inoculation with *B. japonicum* increased these efficiencies compared with the control unioculated treatments. On the other hand, application of FYM decreased these efficiencies obviously, and this may be because the nitrogen in the organic FYM was not readily available for plant and, therefore the soil N (calculated as soil available N plus N applied by fertilizers) and signed as denominator was much lower than the actual values. Similar results were obtained by Reddy *et al.* (1998) who found that *B. japonicum* inoculation recorded higher N recovery than uninocubated plants. Our values which ranged from 10.31 to 28.07 % for NUE and from 8.50 to 22.54% for NPE were somewhat higher than the values of N use efficiency obtained by Caliskan *et al.* (2008) which varied between 2.73-12.63 % and the within the values of N uptake efficiency obtained by George and Singleton (1992) which varied between 16-49 % for soybean at physiological maturity

Table (6): Effect of applied N rate and source as well as inoculation with *B. japonicum* on the values of nitrogen use efficiency (NUE), nitrogen uptake efficiency (NPE) and nitrogen harvest index (NHI) (Data are the mean values of two seasons)

Treatments	Nitrogen use efficiency NUE	Nitrogen uptake efficiency NPE	Nitrogen harvest index NHI (%)
T1	20.94	9.26	62.06
T2	16.46	13.66	69.63
T3	25.47	16.05	70.18
T4	28.07	22.54	70.00
T5	12.52	8. 50	74.55
T6	10.31	9.04	71.46

Uninoculated control treatment (T_1), uninoc.+167 kg N ha^{-1} as MNF (T_2), inoc. (T_3), inoc.+48 kg N ha^{-1} as MNF (T_4), inoc. + 24m^3 FYM ha^{-1} (T_5) and inoc. + 48m^3 FYM ha^{-1} (T_6).

8. Effect of inoculation and nitrogen sources on nitrogen availability in soil and some soil properties

Results in Table 7 demonstrate that N availability in soil at the end of the growing seasons increased with *B. japonicum* inoculation and that the application of FYM had caused further increases in N availability in soil. Although, the added amounts of applied N were lower in FYM applications than mineral N-fertilizers, yet the release of N from FYM might be relatively slower and this ensured the presence of higher concentrations of available N in soil all over the growth period of soybean.

No significant effect of inoculation with *B. japonicum* was noticed on soil pH except for the soil that received FYM applications and further reduction in soil pH was noticed with increasing the applied rate of FYM. The decrease of soil pH with FYM application could be related to the dissociation of the carboxylic groups resulted from the decomposition of FYM in soil (Yan *et al.*, 1996).

Also, the application of FYM increased significant soil total porosity, with no significant effect of inoculation on soil porosity, and this may be related to the formations of soil aggregations by the organic matter applied to soil. This result agrees with those of Haynes and Naidu (1998). Furthermore, the stability of these aggregates against disruptive forces depends also on the organic matter in soil (Oades, 1984).

Table (7): Soil available N, soil pH and total porosity as affected by inoculation and nitrogen sources (Data are the mean values of two seasons)

Treatments	Available N (mg kg^{-1})	pH values (1:2.5) soil: water suspension	Total porosity (%)
T1	22.75f	8.22a	50.20c
T2	24.55e	8.21a	50.75c
T3	42.9d	8.23a	50.75c
T4	48.3c	8.23a	51.10c
T5	70.8b	8.05b	53.95b
T6	80.6a	7.85c	56.75a

Uninoculated control treatment (T_1), uninoc.+167 kg N ha^{-1} as MNF (T_2), inoc. (T_3), inoc+48 kg N ha^{-1} as MNF (T_4), inoc. + 24m^3 FYM ha^{-1} (T_5) and inoc. + 48m^3 FYM ha^{-1} (T_6).

CONCLUSION

Generally, it could be concluded that FYM application at the rate of 48 m^3 ha^{-1}+ biofertlizer inoculation (complete substitution for MNF) could be recommended for high crop yield production and maintaining good soil properties. Application of low dose of MNF (48 kg N ha^{-1}) + biofertlizer inoculation showed relatively similar effect to that of the recommended dose of MNF 167 kg N ha^{-}. Thus, these treatments can replace partially or even completely the high application dose of MNF 167 kg N ha^{-1} for soybean production.

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ARABIC SUMMARY

التأثير المتكامل للنيتروجين المعدنى و التسميد الحيوى والعضوى علي إنتاجية محصول فول الصويا

للسادة الدكاترة

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أجريت هذه الدراسه لتقييم أثر الإحلال الجزئى أو الكلى للأسمده الأزوتيه المعدنيه بإستخدام التلقيح الحيوى منفردآ أومختلطآ بأقل جرعه تنشيطيه من السماد الأزوتى المعدنى أو مختلطآ مع معدلين من الأسمده العضوية (24 – 48 م^3 / هكتار) كمصدر للأزوت ومقارنة ذلك بالتسميد الأزوتى المعدنى الموصى به (167 كجم / هكتار). وذلك من خلال إجراء تجربه حقليه على مدار موسميين زراعيين صيفيين متتاليين لمحصول فول الصويا (2009/2010) .

هذا وقد تم تقدير التغيرات الناشئه فى التربه والمحصول عن اثر هذه المعاملات ومنها على سبيل المثال :– رقم حموضة التربه بعض العناصر الغذائيه المتيسره والمساميه الكليه ونشاط انزيم تثبيت الأزوت الجوى والتعداد الميكروبى وتنوعه فى منطقة الريزوسفير، ومعايير النمو المحصولى ومحتوى النبات من الأزوت والفوسفور والبوتاسيوم فى المراحل المختلفه لنمو النبات وكذلك مكونات المحصول بالإضافه الى كفاءة إستخدام النبات للسماد الأزوتى .

وتشير النتائج المتحصل عليها من تقييم هذه المعاملات الى مايلى :–

التلقيح الحيوى لبذور فول الصويا بصفه عامه قد ادى الى زيادة عدد العقد الجذريه للنبات وكذلك وزنها الجاف وزيادة النشاط الإنزيمى لتثبيت الأزوت الجوى وكذلك التعداد الكلى للميكروبات مقارنه بمعاملة التسميد الأزوتى المعدنى بالمعدل الموصى به ، وقد ادت زيادة المعايير السابقه الى زيادة محتوى البذور والقش من النيتروجين والفسفور والبوتاسيوم وبالتالى تحسين محصول فول الصويا وجودته . وقد اظهرت معاملة الإحلال الجزئى للتسميد المعدنى " والتى تميزت بأعلى قيم لقياس كفاءة إستخدام وإمتصاص عنصر النيتروجين (27.21 و28.92 على التوالى) مقارنه بالمعاملات موضع الدراسة " تأثيرآ مماثلآ نسبيآ بمعاملة التسميد الأزوتى المعدنى بالمعدل الموصى به فيما يختص بمحصول فول الصويا (قش وبذور) وكذلك معايير نمو المحصول ومحتواه من عناصر النيتروجين والفسفور والبوتاسيوم .

على الجانب الآخرأظهرت المعامله المختلطه من التلقيح الحيوى والسماد العضوى بمعدل 48 م^3 هكتار" والتى تمثل إحدى خيارات الإحلال الكامل للأسمده الأزوتيه المعدنيه قيد الدراسه " على مدار الموسمين افضل النتائج وأعلاها معنويه فيما عدا معايير كفاءة استخدام وامتصاص الأزوت) فيما يختص بالمعايير السالفة الذكر والتى ترتبط بالتربه والنبات .