



Impact of interaction between organic nitrogen and Bio fertilizers on quality and productivity of Pea (*Pisum sativum* L.) plants

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Abstract : Using organic and bio fertilizers can make as an alternative to use mineral fertilizers. The nitrogen fertilizer requirement of pea plant is high, reverse all the legumes plants, because it is less in the fixation of atmospheric N. Two field experiments were carried out in factorial randomized complete block design with three replicates. Each experiment included nine treatments, two rates of chicken manure (ChM) as a source of organic N; 80 and 100 kg N fed⁻¹ applied singly or in combined with three kind of bio fertilizer (Nitrobin, Biogen and Halex-2). The application of 100 kg N fed⁻¹ as chicken manure + Nitrobin gave the highest values of N₂-ase activity (86.49) and all nodules characters i.e. nods number/plant and nod fresh and dry weight (20.44,0.471,0.045) Moreover, inoculating seed of pea by all types of bio fertilizer have significantly increased in all studied parameters. The highest content of protein and carbohydrate (25.17and16.05) were obtained when plants fertilized with100 kg N fed⁻¹ as chicken manure + Nitrobin. The seed yield of pea plants was highly significant effect with addition of 100 kg N fed⁻¹ as chicken manure + Nitrobin, which considered as one of the effective agriculture practices in organic farming and environmentally safe procedure.

Key words:Chicken manure, bio-fertilizer, pea plant, growth, pods yield and quality.

Introduction

Using organic fertilizers; chicken manure and bio fertilizer can serve as an alternative practice to use N mineral fertilizers¹, which play an important role in improving soil physical properties². It contributes in increasing the organic soil carbon content and raising soil productivity, through an increase in activity of the useful microorganisms in the soil^{3,4}. Convert organic nutrient's forms to mineral forms, which become available to plants as the slow-release fertilizers⁵. Moreover, chicken manure contains higher levels of relatively available nutritional elements, especially N, which is essentially required for plant growth⁶.

Bio fertilizer use become an important component in an integrated nutrient supply system and hold a great promise to improve crop yields through better nutrient supplies. *Azotobacter*, *Azospirillum* and *Klebsiella* are the most important bacteria to the non-symbiotic fixation of atmospheric N. Biofertilizer plays a vital role in restoring the natural soil nutrient cycle by fixing and released plant available N forms to soil⁷, as well as stimulating plant growth through the synthesis of growth promoting substances⁸. Nevertheless, the main mechanism of this beneficial effect is not fully understood. Among the explanations proposed for plant growth promotion by these bacteria is an increased uptake of mineral nutrients⁹. In addition, ¹⁰indicated that inoculated plants significantly surpassed un-inoculated ones in number of nodules/plant, plant height, number of branches and pods/plant, seed yield/plant, 100-seed weight and seed yield/fed. On the other hand, the number of seed/pod, pods weight/plant, seed N content % and seed protein content % were not affected by inoculation. pea

(*Pisum sativum L*) is one of the foremost important versatile legume crop which is highly nutritious due to its important bio-chemical attributes viz protein content, protein quality (having good amount of essential amino acids such as lysine, methionine, leucine etc which are not synthesized by the human body), minerals, oils and sugar content. Peas are highly nutritive and contain a high percentage of digestible 22.5% proteins, 58.5% carbohydrates, 1.0% fats, 4.4% fibers and 3% minerals vitamins, particularly of the B group¹¹.

Therefore, the present study aimed to evaluate the combined effect of chicken manures and three isolates of bio fertilizer on pea plants growth, chemical composition and green pod yield and its components as well as dry seed yield and seeds quality. Such study aimed also to decrease the pollution resulting from using mineral fertilizers by the application of bio fertilizers and encourage the organic production of pea in Egypt.

Materials and Methods

Two field experimental designs was carried out at the private farm, Shibeen El Knatr, El Qualubia Governorate, Egypt during two successive seasons 2014 and 2015. to study the effect of the interaction between organic fertilizer (chicken manures) and three isolates of biofertilizer as Nitrobin, biogen and Halex-2 at a rate of 2 kg fed⁻¹ application on growth, yield and pods quality of pea (*Pisum sativum L.*) cv. Master B. Chicken manure and bio fertilizer were applied during preparing the soil for sowing.

Treatments were arranged in factorial randomized complete block design with three replicates. Seeds of pea were sown on 20 November in 2014 and 2015, respectively. Seeds were sown on one side of ridges, ridges was 80 cm width and 4 m length and 25 cm apart. Each plot included 4-ridges and the plot area was 12.8 m². Experimental soil was subjected to mechanical and chemical analyses according to the method described by¹². Characterization of the investigated chicken manure were 3.3, 1.2, 1.1 and 5.8% for NPK and moisture content, respectively, and its amounts equivalent to each N rate (ton fed⁻¹) in the two seasons it was 2.604 and 3.208 at 80 and 100 kg N fed⁻¹. The physical and chemical analyses were presented in Table 1. The normal agricultural practices required for pea production were applied as commonly followed in the farm. The chemical fertilizers (NPK) were used as recommended full dose 100% NPK (100 kg N fed⁻¹ as ammonium sulfate, 30 kg P₂O₅ fed⁻¹ as calcium superphosphate and 50 kg K₂O fed⁻¹ as potassium sulfate). The chemical analysis of chicken manure was presented in Table 2.

Table 1: Physical and chemical properties of the soil experimental site in two seasons

Particle size distribution			Texture classes	pH (1:2.5)	EC (dS m ⁻¹)	O.M (g kg ⁻¹)	Macronutrient available (mg kg ⁻¹)		
Sand (%)	Silt (%)	Clay (%)					N	P	K
12.25	30.51	56.54	Clay	8.21	1.16	15.23	40.21	9.27	382

Data recorded:

Vegetative growth: A random sample of 3 plants from each plot was taken at 50 days after sowing and the following vegetative characters were recorded: plant length, number of leaves and number of shoots as well as fresh and dry weight of leaves and shoots.

Nodulation Status: Six plants from each plot were taken at random after 50 days from sowing to evaluate the number of nodules/plant, fresh and dry weight of nodules (g). Nitrogenase activity was estimated in nodules using an acetylene reduction technique according to¹³ as $\mu\text{l C}_2\text{H}_4/\text{g dry nodules}$.

Pods yield: At harvest stage the mature pods of pea for each experimental plot were collected along the harvesting season and the total pods yield was recorded as ton fed⁻¹.

Pods quality: Random sample of 50 pods from each plot was taken and the physical properties (average pod weight, length and seeds weight/pod).

Chemical constituents i.e. N, P, K, Mn, Fe, total protein and total carbohydrates were recorded in pea pods. Total nitrogen, phosphorus and potassium were determined according to the method described by¹⁴. However, Mn and Fe-content was determined using flame ionization atomic absorption spectrometer model 1100 B of Perkin Elemer according to the method of ¹⁵. The protein percentages in pods were accounted by multiplying nitrogen content by 6.25. Total carbohydrates were determined according to the methods mentioned by¹⁶. The physical and chemical properties of the soil were determined according to¹⁷. All data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the Completely Randomized Design (CRD) using MSTATC computer software package according to¹⁸.

Results and Discussion

Vegetative growth

Data in Table (2) indicated that the lowest records of pea growth characteristics, i.e. plant length, number of leaves and buds per plant, fresh and dry weight of leaves and buds were observed with application of chemical fertilizers. While, the combination with organic and bio fertilizers shows the significant increase of mentioned studies. Generally, the higher values of all estimated parameters gave with application of 100 kg N fed⁻¹ chicken manure+ Nitrobin (69.94, 17.74, 3.33, 68.95, 56.33, 23.52 and 14.69). This might be due to the slow release of nutrients from organic manures, and when supplemented with inorganic fertilizers it might have helped the microorganisms in the faster decomposition of organic manures, thereby increasing the availability of nutrients, especially nitrogen, which helps in protein synthesis and ultimately resulting in higher plant growth rate, more leaves and branches. These results are consistent with the findings of¹⁹ in Faba Bean, and²⁰ in pea.

Table 2: Vegetative growth of pea plants as affected by organic and bio fertilizer

Treatments	Plant length (cm)	Number of		Fresh weight (gm)		Dry weight (gm)	
		leaves	Buds	leaves	Buds	leaves	Buds
NPK (Control)	43.65	8.62	1.81	40.23	41.76	11.70	8.17
80 kg N fed⁻¹ as ChM	43.16	8.23	1.96	39.95	42.58	11.28	8.37
100 kg N fed⁻¹ as ChM	45.51	11.41	2.38	42.17	48.18	13.24	10.30
80 kg N fed⁻¹ as ChM + Nitrobin	58.34	15.62	2.90	60.89	52.46	18.50	12.54
100 kg N fed⁻¹ as ChM+ Nitrobin	69.94	17.74	3.33	68.95	56.33	23.52	14.69
80 kg N fed⁻¹ as ChM+ Biogen	57.59	14.61	2.46	56.16	50.50	17.01	11.68
100 kg N fed⁻¹ as ChM+ Biogen	67.85	16.56	3.11	62.79	53.74	21.83	13.55
80 kg N fed⁻¹ as ChM+Halex-2	55.21	13.69	2.27	53.87	49.83	16.45	11.24
100 kg N fed⁻¹ as ChM+Halex-2	66.02	15.86	2.88	61.64	51.9	21.52	12.47
L.S.D 5 %	0.673	0.311	0.142	0.462	0.667	0.390	0.389

ChM: chicken manure

Yield and its components of Peadata in Table (3) indicated that the all estimated pea yield parameters, i.e. Pod Length, weight of pod (g), number of seed/pod and weight of seed/pod (g) were significantly affected by all applied treatments in this study during the two growing seasons. The highest values of all parameters were indicated by application of 100 kg N fed⁻¹ chicken manure+ Nitrobin (10.65, 5.21, 35.95 and 2.38). May be, there More plant height and number of leaves per plant and number of branches per plant increased the photosynthetic area and favorable physiological activities which resulted in the production of more number of pods per plant, pod length and pod weight, these findings corroborated the earlier reports of^{21, 22 and 23}, Highest number of pod per plant and maximum numbers of seeds per pod were observed with application of organic with bio fertilizer. These findings are agreement with the findings of^{24, 25, and 26}.

Table3: Pea total green pods yields as affected by organic and bio fertilizer

Treatments	Pod length (cm)	Pod weight (g/pod)	Seeds no per pod	Seeds weight (g/pod)	Total yield (Ton/fed.)
NPK (Control)	6.79	3.157	11.00	1.267	2.35
80 kg N fed ⁻¹ as ChM	7.057	3.31	11.45	1.45	2.33
100 kg N fed ⁻¹ as ChM	8.98	3.88	13.74	1.81	2.88
80 kg N fed ⁻¹ as ChM + Nitrobin	9.85	4.31	24.15	2.09	3.62
100 kg N fed ⁻¹ as ChM+ Nitrobin	10.65	5.21	35.95	2.38	4.17
80 kg N fed ⁻¹ as ChM+ Biogen	9.47	4.14	22.68	1.97	3.35
100 kg N fed ⁻¹ as ChM+ Biogen	10.28	5.03	31.84	2.22	4.08
80 kg N fed ⁻¹ as ChM+Halex-2	9.27	4.08	21.14	1.84	3.18
100 kg N fed ⁻¹ as ChM+Halex-2	10.19	4.9	30.28	2.16	3.91
L.S.D 5 %	0.134	0.116	0.630	0.084	0.107

ChM: chicken manure

It is revealed from the data obtained (Table 3) that a significantly maximum yield of 4.17Tonfed⁻¹ was obtained in pea under the treatment of 100 kg N fed⁻¹ such as chicken manure+ Nitrobin. Similarly,^{27, 28,29} noted that the integrated application of chemical fertilizers in conjunction with manures and bio fertilizers was the best option. The integration of biofertilizers along with chemical fertilizers and FYM not only has a positive effect on the yield attributes of pea, but also in the process leads to the highest net returns²⁴. Application of mineral nitrogen fertilization combination with bio-fertilizer had a positive effect on increased yield in olive trees³⁰

Chemical Constituents of Pea Seeds

Results are presented in Table (4) indicated that the chemical constituents of pea seeds as a response of organic and bio fertilizer. Data showed that the highest values of all estimated parameters, i.e. N, P, K, Mn and Fe(4.16%,0.53%, 2.27%, 33.28 ppm and 509.52 ppm) in pea seeds were observed in plants application of 100kg Nfa⁻¹ as chicken manure+ Nitrobin. In this respect,^{31,32} reported that Nitrogen, Phosphorus and Potassium contents in leaves of pea significantly increased with *Rhizobium* as compared with the control. These results confirmed with,³³ mentioned that application of FYM contains microorganisms has that ability to supply plants with fixed N,P and release phytohormones which could increase the growth and dry weight and in turn increase NPK and content in tissues of tomato plants.

Table 4: Effect of organic and bio fertilizer on chemical constituents of Pea pods

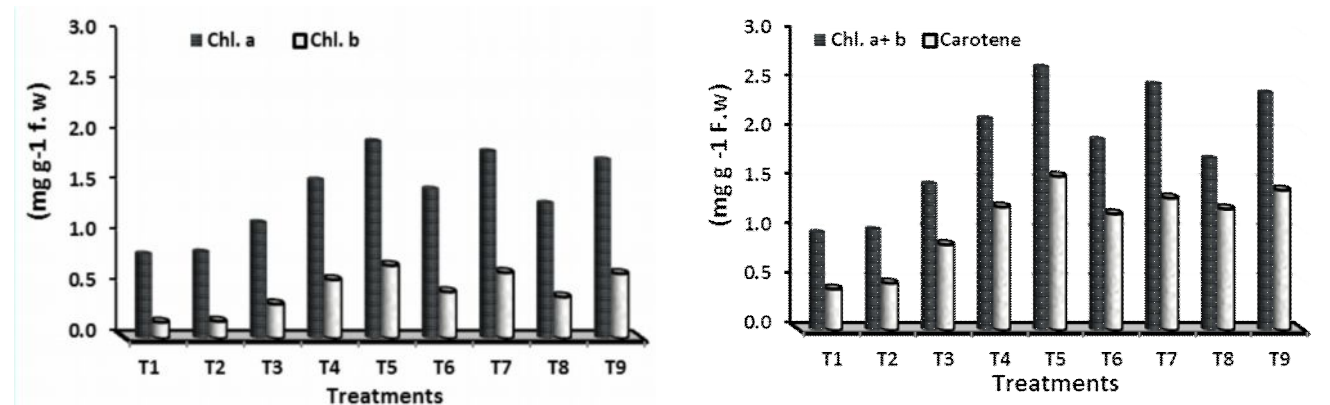
Treatments	Macronutrient (%)			Mn (ppm)	Fe (ppm)	Total protein (%)	Carbohydrate (mg/100gm seeds)
	N	P	K				
NPK (Control)	2.27	0.30	1.47	25.14	307.9	14.19	13.02
80 kg N fed ⁻¹ as ChM	2.41	0.35	1.69	27.29	391.9	14.51	13.18
100 kg N fed ⁻¹ as ChM	2.78	0.45	1.95	29.18	426.2	16.93	14.37
80 kg N fed ⁻¹ as ChM + Nitrobin	3.60	0.51	2.09	30.22	468.4	22.28	15.04
100 kg N fed ⁻¹ as ChM+ Nitrobin	4.16	0.53	2.27	33.28	509.5	25.17	16.05
80 kg N fed ⁻¹ as ChM+ Biogen	3.20	0.47	2.07	30.18	437.4	21.85	14.85
100 kg N fed ⁻¹ as ChM+ Biogen	3.79	0.52	2.14	32.39	493.4	24.47	15.55
80 kg N fed ⁻¹ as ChM+Halex-2	3.07	0.43	1.96	29.66	430.0	20.14	14.38
100 kg N fed ⁻¹ as ChM+Halex-2	3.61	0.47	2.13	31.88	482.4	23.29	15.19
L.S.D 5 %	0.126	0.033	0.072	0.079	8.464	0.164	0.120

ChM: chicken manure

On the other hand, data in table 4 showed that the mean values of total protein content and carbohydrate slightly differed in all treatment. The highest content of protein and carbohydrate (25.17 and 16.05) were obtained when plants fertilized with 100 kg N fed⁻¹ as chicken manure + Nitrobin. This may be due to the synergistic effect between all applied microorganisms. These results were in harmony with the results obtained by ³⁴. It was also noted that the lowest value of phosphorus and potassium in pea seeds in plants fertilized with NPK only.

Photosynthetic pigments

It is clear from Fig (1) that, the combination of organic nitrogen with biofertilizer increases the content of photosynthetic pigments (chlorophyll a, b, a+b and carotenoids). The highest values were obtained from the application of using 100 kg N fed⁻¹ as chicken manure + Nitrobin. These results are confirmed by those recorded by ^{35,36} worked on pea plants. This can be attributed to the sufficient N uptake, enhanced onion plants to absorb more N and in turn to build more chlorophyll structure. It is concluded that application of chicken manure using 100 kg N fed⁻¹ and inoculation the seed of pea plant with bio fertilizer (Nitrobin) was leading to increase the growth parameters, yield and its components.



T1:NPK (Control)	T4:80 kg N fed⁻¹ ChM + Nitrobin	T7: 100 kg N fed⁻¹ ChM + Biogen
T2:80 kg N fed⁻¹ ChM	T5:100 kg N fed⁻¹ ChM + Nitrobin	T8:80 kg N fed⁻¹ as chicken manure + Halex-2
T3:100 kg N fed⁻¹ ChM	T6:80 kg N fed⁻¹ ChM + Biogen	T9:100 kg N fed⁻¹ as chicken manure + Halex-2

Fig (1): Chlorophyll a, b, a+b and carotene content of Pea as affected by organic and bio fertilizer

Nitrogenase activity and nodules characters

Data in Table (5) show the N₂-ase activity in nodules was affected by all investigated treatments. It was clearly that pea plants treated only with chemical fertilizers gave lower values of N₂-ase activity than plants treated with organic and bio fertilizers. This result is in harmony with that obtained from ³⁷ who demonstrated that the addition of chemical fertilizers decreased of nitrogenase activity.

Table 5: Effect of organic and bio fertilizer on Nitrogenase activity and nodules characters in root of pea plant.

Treatments	Nitrogenase activity as $\mu\text{l C}_2\text{H}_4/\text{g dry nodules}$	Nodules number/plant	Nodules Fresh weight (gm)	Nodules Dry weight (gm)
NPK (Control)	23.07	1.41	0.123	0.003
80 kg N fed⁻¹ as ChM	23.28	3.13	0.133	0.003
100 kg N fed⁻¹ as ChM	33.66	4.9	0.160	0.004
80 kg N fed⁻¹ as ChM + Nitrobin	73.93	13.44	0.260	0.031
100 kg N fed⁻¹ as ChM+ Nitrobin	86.49	20.44	0.471	0.045
80 kg N fed⁻¹ as ChM+ Biogen	66.48	12.71	0.220	0.026
100 kg N fed⁻¹ as ChM+ Biogen	79.09	19.77	0.443	0.050
80 kg N fed⁻¹ as ChM+Halex-2	61.22	11.81	0.190	0.019
100 kg N fed⁻¹ as ChM+Halex-2	77.25	18.77	0.367	0.033
L.S.D 5 %	0.460	0.206	0.022	0.112

ChM: chicken manure

The highest significant values of N₂-ase activity were observed in nodules formed in plants inoculated with bio fertilizers in combination with organic nitrogen. 100 kg N fed⁻¹ as chicken manure + Nitrobin gave the highest values of N₂-ase activity (86.49). This result may be due to the activities of inoculate as well to the synergistic effect between inoculate and organic matter. These results are agreement with³⁸ who found that pea plant inoculated with *R. leguminosarum* and *Glomus* spp. gave a significant increase in N₂-ase activity. Also,³⁹ reported that foliar spraying of pea plant with bio fertilizers improved enzyme activities. In this respect, ⁴⁰ proved that mycorrhizal colonization with *G. mosseae* combined with *R. leguminosarum* increased the enzyme activities of pea plants. Also, data in table 5 show the effect of organic and bio fertilizer on nodules characters in root of pea plants during the two winter seasons. In this respect, data indicated that all nodules parameters, i.e. nods number/plant and nod fresh and dry weight were lower in plants fertilized with chemical fertilizers than plants inoculated with bio fertilizers either in the presence or absence of organic matter application. The highest values of all nodules characters were observed in the case of plants addition 100 kg N fed⁻¹as chicken manure + Nitrobin. These results are consistent with ⁴¹ who found that co-inoculation of pea plants with bio fertilizers result in significant increase in nodule number, nodule fresh weight. Also,⁴² reported that the inoculation of garden pea with rhizobia increased nodule numbers and weights and nodule dry matter.

Conclusion:

Obtained results indicated that growth performance and yield of pea were significantly affected by application of biofertilizers in combination with organic nitrogen. In addition, results proved that using of 100 kg N fed⁻¹ as chicken manure + Nitrobin leading to increase of all parameter.

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