Effect of Organic, Inorganic N Sources and *Rhizobium* Inoculation on Yield Performance and N Uptake Faba Bean Grown in A Calcareous Soil

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KEY WORDS: FYM, AN, *Rhizobium*, faba bean, yield, N uptake, calcareous soil

ABSTRACT

A field trail was carried out to compare the single and combined effects of farmyard manure (FYM), ammonium nitrate (AN) and *Rhizobium* inoculation on yield performance and N uptake by faba bean grown on a calcareous soil. All treatments were arranged in a split-plot design with three replicates. Both fertilizers were addressed at a rate of 30 kg N/ fed. At fruiting and late ripe stages, random plants were sampled to evaluate the nodulation index, yield performance and N uptake.

The results have shown that FYM promoted the nodulation index, expressed as nodules number and their massive growth, than AN-treated plants. The relative comparison between N-supplying sources proved that the advantages of FYM accounted for 67.9 and 82.9% for the respective trails. The corresponding benefits of *Rhizobium* treatment averaged 16.9% for the nodulations number and 5.1 for its weight. Similar trend was also noted on faba bean yield and its components, except the weight of 100-seed, but the response rate varied considerably among the studied trails. Relative to AN treatment, the stimulatory effects of FYM addition were, subsequently, 8.5, 17.4 and 5.5% for total biomass, seed yield and seed protein percentage. The comparable advantage, due to inoculation treatment averaged 8.7, 28.3 and 15.4% on the respective trails. Except the straw yield component, documented data showed marked significant differences on dry matter yield with variable degree of variations, due to the main effects of N-supplying sources , inoculation treatments and their interactions. The results reported on N content and its accumulation in pods and whole plant followed up the same trend. Based on the positive results detected on the 2-way interaction of our experimental data, it becomes evident that FYM is more creative to achieve optimal yield records and seed protein, when applied at a rate of about 7.0 ton/fed (30 kg N/fed) to the seed-inoculated faba bean grown in the calcareous soil. The reported data proved that FYM induced considerable reduction in soil reaction and NO_3-N content in the soil rather than AN.

INTRODUCTION

On the management of N use efficiency by plants, attention is being directed to introduce organic and biofertilizer N forms to cropland, as an alternative mean for the popular inorganic N fertilizers, to manipulate N uptake performance and enhancing crop yield potentials. This strategy is apparently warranted, particularly in calcareous and sandy soils, because the application of NH_4^+-N and NO_3^--N fertilizers are subjected to nitrification and subsequent loss by leaching and denitrification, imposing environmental contamination of ground water, rivers and streams. From the economic and environmental concerns, it seems possibly that application of organic fertilizer to calcareous soils becomes essential, owing to its high value as a soil conditioner (Hati et al., 2006 and Im, 1982) and as a rich nutrient support (Daooud, 2005). Evidences have shown that addition of organic matter to soils containing $CaCO_3$ was beneficial not only to alleviate the crust formation that may hamper seed germination and plant growth (Peiter et al., 2000), Ca-induced Mg deficiency (Islam et al., 1987) but also to stimulate P and micronutrient availability (Marschner, 1995 and Tyler, 1992). Besides, it improves the soil physical properties, including soil aggregation, porosity, bulk density, hydraulic conductivity and water retention (Clapp et al., 1986).

Previous studies (Elaskakov et al., 1991 and Hussein and Abdel-Aziz, 1992) demonstrated that organic manure application was successful for increasing plant biomass and yield of field crops, due to the potential release of essential nutrients as a result of bio-chemical reaction (Abdel-Sabour et al., 1997). Evidences have shown that incorporating organic fertilizer with NPK was primitive for releasing more available N than each individual input (Metwally and Khamis, 1998), accompanied with marked increases of faba bean yield (Puponin et al., 1991). Reported data on biofertilizer indicated that symbiotic bacterium and *Rhizoboum* were creative to support legumes with its N need with an attendant increase in total protein (Bedrous et al., 1990)

Based on these facts, the present study is being suggested to evaluate the single and combined effects of farmyard manure (FYM), ammonium nitrate (AN) and *Rhizobium* inoculation on nodulation index, yield performance and N uptake by faba bean grown in the calcareous soil. Besides, the changes in soil pH and NO_3-N content were also assessed to evaluate the side effects of such variables.

MATERIALS AND METHODS

A factorial experiment was carried out in the field on a private farm at El-Fayoum Governorate to evaluate the main effects of organic farmyard manure (FYM), ammonium nitrate (AN) sources and *Rhizobium* inoculation, expressed as symbiotic bacterium (*Rhizobium leguninasarum* cv. *Vicieae bacterium*) on yield performance and N uptake by faba bean (*Vicia faba* L., Giza 2). The soil was a typical calcareous with pH 8.5, sandy clay loam in texture and poor in total N. The chemical characteristics of the selected soil and organic manure composition are given in Table 1.

Moderate N application rate, accounting for 30 kg N/fed for both FYM and AN, was applied to the growing plants. Before planting, the calculated amount of FYM (about 7.0 ton/fed) was incorporated, at once, to the top soil (0 – 30 cm). The amount of AN fertilizer was partitioned into two equal doses, i.e., the first part was addressed before planting and the rest was added two weeks later to compare their reactions in the presence and absence of *Rhizomium* inoculation. In this respect faba bean seeds were treated with inoculum suspension, containing, containing 10^8 cell bacterium per one gram. All treatments were arranged at random in split-plot design with three replicates, considering the N-supplying sources as a main plot and inoclum as sub-plot. Bean seeds were sown in 2007 in plot area, outlined by 5×5 m, keeping the plant stand constant. Besides, superphosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O) fertilizers were provided at rate 15 kg P_2O_5/fed and 24 kg K_2O/fed, respectively. Both fertilizers were addressed before planting.

Table (1): Soil chemical and physical characteristics and farmyard manure (FYM) composition

Parameters	Soil				FYM
pH	8.50				7.90
EC (1:5), dSm^{-1}	4.37				8.20
OM, %	0.81				13.64
Total N, %	0.04				0.44
C/N ratio	11.19				57.65
Available N, mg kg^{-1}	n.d*				353
Available P, mg kg^{-1}	9.4				269
Available K, mg kg^{-1}	387				2800
$CaCO_3$, %	23.80				n.d
Soluble inions meq/l	Cations		Anions		
	Ca^{++}:	1.17	CO_3^{--}:	--	n.d
	Mg^{++}:	0.36	HCO_3^-	1.34	n.d
	Na^+:	2.70	Cl^-:	2.48	n.d
	K^+:	0.21	SO_4^{--}:	0.62	n.d
Particle size distribution**	Sand= 51.2, Silt=20.6, clay=28.2				

* n.d: not determined.
** soil texture class: sandy clay loam (**SCL**).

At Fruiting and late ripe stages (maturity stage), plant samples were collected, whereas the green and dry pods were recorded. At the respective stages, dry matter yield was noted and N content of seeds was determined, according to Chapman and Pratt (1961). At the end of the experiment, random soil samples were collected from the upper soil surface (0-30 cm) of treated plots to follows up the changes in soil pH and NO_3-N concentration, using the standard methods (Black et al., 1982) The data were statistically analyzed, according to Snedecor and Cochran (1981). Simple mathematical calculations were introduced to follow up the beneficial effect of variable treatments on the recorded data.

RESULTS AND DISCUSSION

1- Nodulation index and yield performance:

The ANOVA presented in Table 2 showed that all the recorded data; except the 100-seed weight, were significantly influenced by the main effects of the applied N sources, inoculation treatments and their interaction. Regardless to the inoculation effects, farmyard manure (FYM) imposed greater performance on nodulation index than ammonium nitrate (AN). On average, the stimulatory effects on nodules development and its massive yield accounted for 67.8 and 82.9%, respectively. The results outlined on *Rhizobium* inoculation revealed that this treatment was essential to induce marked increases, accounted for 16.9 and 5.1% on nodules number and weight per plant.

Table (2): Effect of organic (FMY)and ammonium nitrate (AN) fertilizers and *Rhizobium* inoculation on nodulation index and yield of faba bean

Treatments		Nodulation Index#		Yield and yield components				
N - sources	Inoculation (I)	Nodules no/plant	Nodules wt. g/plant	Total biomass kg/fed	Seeds kg/fed	100 seed wt., g	Seed protein %	HI##
FYM	- Inoc.	75.9	2.22	2223	678	124	16.83	30.5
	+ Inoc.	86.9	2.49	2733	984	126	19.13	36.0
	mean	81.4	2.36	2478	831	125	17.98	33.3
AN	- Inoc.	43.9	1.34	2339	669	120	15.69	28.6
	+ Inoc.	53.0	1.25	2227	746	122	18.38	33.5
	mean	48.5	1.29	2283	708	121	17.04	31.1
Overall mean	- Inoc.	59.9	1.78	2281	674	122	16.26	29.6
	+ Inoc.	70.0	1.87	2480	865	124	18.76	34.8
ANOVA (significant level)								
N Source (N)		**	**	*	**	ns	*	*
Inoculation (I)		**	*	*	**	ns	**	*
N × I		**	**	**	**	ns	**	**

\# Nodulation data were recorded after 6-8 weeks of planting data.
\## HI = Seed weight / total top biomass.
* Significant at the 5% level of probability.
** Significant at the 1% level of probability.
ns not Significant at the 5% level of probability.

The results given in Table 2 revealed that, except 100-seed weight trait, FYM was primitive to exhibit marked increases on the yield components than AN. Qualitatively, the calculated profits associated with FYM input were 8.5, 17.4 and 5.5% for the total biomass (above ground plant part), seed yield and seed protein, respectively. The stimulatory effects of inoculation treatment were, subsequently, 8.7, 28.3 and 15.4% on the respective traits. Evidently, when the applied N sources were addressed to the inoculated seeds (N × I), total plant biomass, seed yield and protein% were progressively increased by 22.9, 45.1 and 13.7% for FYM, followed by 0.9, 11.5 and 17.1% for the AN, respectively. Besides, marked significant variations were also recorded on harvest index (HI) criteria, due to N × I interaction. These results proved that FYM application was more superior than AN fertilizer for increasing seed yield, due to the continuous N supply and slow N released from organic manure that acted well for massive nodulation (Hadvizdeh and George, 1989, and Voss et al., 1987). On the other hand, the relative drop in seed yield associated with AN fertilizer could be ascribed to the excessive available N in rooting zone in amounts exceeded the critical limit required for activating the nodulation process. Reported data (Kaushik et al., 1993; Liebhard, 1989 and Minar and Zehnalek, 1989) agree quite closely with our experimental data

2- Dry yield components at fruiting and late ripe stages:

The data given in Table 3 showed that the main effects of N - supplying sources, *Rhizobium* inoculation and their interactions imposed marked significant variations on dry matter yield of pods and total biomass of whole plant at fruiting and late ripe stages. The results outlined on straw yield, associated with single and combined treatments, revealed insignificant trends. Once again, faba bean provided with FYM exhibited greater performance on pod development and plant growth than AN treatment. Relative to the addressed AN fertilizer, the advantages originated from FYM on pods were 15.4 and 12.4%, respectively, at fruiting and late rip stages. The respective increases on total plant biomass were, subsequently, 5.7 and 12.5%. Similarly, *Rhizobium*-treated plants yielded more pods, accounted for 12.1 and 17.8%, at the respective stages than the control treatment. The comparable increases on total plant biomass were relatively higher (27.4%) at fruiting than the late ripe stages (12.2%).

Table (3): Effect of organic (FMY) and ammonium nitrate (AN) fertilizers and *Rhizobium* inoculation on dry yield components(g/plant) during fruiting and late tripe stages

Treatments		Fruiting stage			Late ripe stage		
N - sources	Inoculation (I)	Pods	Straw	Whole plant	Pods	Straw	Whole plant
	- inoc	135	326	461	258	333	591
FYM	+ inoc	165	344	509	322	345	667
	mean	150	335	485	290	339	629
	- inoc.	129	328	457	245	318	563
AN	+ inoc.	131	330	461	271	330	601
	mean	130	329	459	258	324	582
Overall	-inoc.	132	327	459	252	326	578
mean	+inoc.	148	337	585	297	338	635
ANOVA (significant level)							
N Source (N)		ns	*	**	**	ns	**
Inoculation (I)		ns	**	*	*	ns	**
N × I		ns	**	**	**	ns	ns

* Significant at the 5% level of probability.
** Significant at the 1% level of probability.
ns not Significant at the 5% level of probability.

The data recorded on the two way interactions revealed that the applied FYM to *Rhizobium*-treated seeds was promising to induce marked significant increases on dry matter yield components than AN×I treatment (Table 3). It is worthy to point out that although straw yield was not significantly influenced by N × I interaction; there are marked deviations due to organic and inorganic N input along the inoculation treatment. These results proved that FYM applied to the inoculated treated seeds was impressive not only to accelerate the nodulation process, but also to provide additional N supply, from the mineralization process to meet the N-demand of faba bean. In this regard, similar results were reported by El-Fayoumy and Ramadan (2002), indicating the advantage of organic manure fertilizer in the presence of *Rhizobium* inoculation.

3- Nitrogen content and uptake at fruiting and late ripe stages:

Since the N content, specifically in leaves and stems, are mostly translocated to seeds up to the late ripe stage (Table 4), particular attention is being given to the changes in pod N content when organic and/or inorganic N sources are addressed in the presence of *Rhizobium* inoculation. This might explain the disregard of N uptake calculations in straw and whole plant at that stage. However, the results reported on N performance in plant parts at fruiting and late rip stages, indicated that N content and its accumulation (uptake) in pods and even in whole plant were significantly affected by the single and combined treatment variables, whereas the reverse was true for the straw yield (Table 4).

Table (4): Effect of N fertilizers sources and *Rhizobium* inoculation on N content and its uptake in plant parts (mg /plant) at fruiting stage and N content pod at lat ripe stage of faba bean

Treatments		Fruiting Stage					Lat ripe stage
		Pod		Straw		Whole Plant	
N-sources	Inoculation (I)	N%	Uptake mg/plant	N%	Uptake mg/plant	Uptake mg/plant	N% Pods
FYM	- Inoc.	4.70	634	2.90	945	1579	4.30
	+ Inoc.	4.90	808	2.96	1018	1826	4.90
	mean	4.80	721	2.93	982	1703	4.60
AN	- Inoc.	4.00	516	2.88	934	1450	4.20
	+ Inoc.	4.50	590	2.90	957	1547	4.50
	mean	4.25	553	2.89	951	1486	4.35
Overall mean	-Inoc.	4.40	575	2.89	945	1520	4.25
	+Inoc.	4.70	699	2.93	988	1687	4.70
ANOVA (significant level)							
N Source (N)		**	**	ns	ns	**	**
Inoculation (I)		*	*	ns	ns	**	**
N × I		**	**	ns	ns	*	*

* Significant at the 5% level of probability.
** Significant at the 1% level of probability.
ns not Significant at the 5% level of probability.

Relative to AN treatment, the advantages in pod N content, due to FYM application averaged 12.9 and 10.2% at fruiting and late ripe stages. Similar trend was also detected on the benefits associated with *Rhizobium* treatment, whereas the attendant increases in N contents in pods approached 6.8 and 8.0% at the respective stages. The data of N uptake followed up the same trend, but the deviations were quite different. Such variations are basically inferred to the unequal effect of single and combined treatments on N assimilation and dry matter yield.

The results noted on the two way interaction, confirmed the significant value of FYM when applied to seed-inoculated faba bean to achieve optimal results. On average the increase in N% in pods at late ripe stages accounted for 14.0 and 7.1% when FYM and AN were applied in the presence of *Rhizobium* treatment.

4- Soil pH and NO_3^--N concentration:

The results given in Table 5 proved that FYM induced remarkable reduction in soil pH rather than AN. The deviations in soil reaction between N-supplying sources and initial soil pH (Table 1) were 0.6 and 0.2 pH units, respectively. The changes in soil pH associated with inoculation treatment were not significant. With respect to N×I interaction, FYM exhibited greater acidification than AN in the presence of *Rhizobium* inoculation. The limited variation in soil pH due to the single and combined treatment effects could be reasoned to the high buffering capacity of the calcareous soil, which is mainly controlled by $CaCO_3$%, redox potential of Fe, Al-oxides, organic matter and clay fraction contents.

Table (5): Effect of organic (FMY) and ammonium nitrate (AN) fertilizers and *Rhizobium* inoculation on soil pH and NO_3-N content at the end of experiment

Treatments		pH	NO_3-N %
N-sources	Inoculation		
FYM	- Inoc.	8.0	16.2
	+ inoc.	7.7	14.0
	mean	7.9	15.1
AN	- Inoc.	8.4	22.9
	+ Inoc.	8.2	18.3
	mean	8.3	20.6
Overall mean	-Inoc.	8.2	17.3
	+Inoc.	8.0	18.5
ANOVA (significant level)			
N-Source (N)		*	**
Inoculation (I)		ns	*
N × I		**	**

* Significant at the 5% level of probability.
** Significant at the 1% level of probability.
ns not Significant at the 5% level of probability.

The results detected on NO_3^--N content in soil indicated that its level was actually dropped by 39.7% in FYM, as compared with AN treatment. The comparable variation on NO_3^--N level, due to the inoculation effect averaged 6.9%. The interaction study of N × I variables proved that, across the inoculation treatments, NO_3^--N level in the soil was clearly decreased for FYM rather than AN treatment. On average the reduction in NO_3^--N levels between FYM and AN in the absence of inoculation accounted for 29.2%. The corresponding variation in the presence of inoculation averaged 23.5%. Obviously, the significant variations existed between NO_3^-

-N levels, due to the main and combined treatment effect might explain the changes in soil reaction. In other words, the gradual slow release of NO_3^--N, which is librated from FYM decomposition, acted well not only for optimal nodulation, but also greater N uptake and utilization by faba bean with slight NO_3^--N accumulation in rooting zone. In contrast, opposite results were noted on AN treatments. It seems possibly that the variation NO_3^--N level in soil, are operative for extensive and intensive root exploration, accompanied with more *Rhizosphere* acidification, due to organic acids excretion by plant-root system. These results are in agreement with the data reported by Abdel-Aziz et al. (1996) and Naramabuye et al. (2008).

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Prof. Dr. / Magdy F. Soliman, Soil Salinity Research Department, Soil, Water and Environment Research Institute, for his contractive criticism and reviewing this article.

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الملخص العربي

تأثير مصادر النيتروجين العضوية والمعدنية والتلقيح البكتيري على أداء محصول وإمتصاص النيتروجين لنبات الفول البلدى النامى فى أرض جيرية

رأفت نظمى زكى و آمال وليم أبو الخير و نادر رمزى حبشى
معهد بحوث الأراضى والمياه والبيئة_مركز البحوث الزراعية_الجيزه_مصر

أجريت تجربة حقلية لمقارنة تأثير كل من مكمور مخلفات المزرعة و سماد نترات النشادر والتلقيح البكتيري علي أداء المحصول و أمتصاص النيتروجين لنبات الفول البلدي النامي في أرض جيرية بأستخدام القطاعات المنشقة وكررت جميع المعاملات بثلاثة مكررات. تم أضافة نوعى السماديين بمعدل 30 كجم نيتروجين للفدان وأخذت عينات النبات عشوئيا من خلال مرحلتي الأثمار و النضج التام وذلك لتقييم دليل نمو العقد الجذرية وأداء المحصول وأمتصاص النيتروجين.

وأظهرت النتائج أن مكمور مخلفات المزرعة أدي إلي تنشيط دليل العقد الجذرية من حيث عددها ووزنها بالمقارنة بسماد نترات النشادر. برهنت المقارنات النسبية بين أضافة المصادر المختلفة من الأسمدة الأزوتية أن هناك تفوق لمكمور مخلفات المزرعة بمقدار 67.9 ،82.9% خاصة بالنسبة للمعاملات السابقة. أوضحت نتائج التلقيح البكتيري زيادة بلغت فى المتوسط 16.9% بالنسبة لعدد العقد الجذرية و 5.1 بالنسبة لوزنها. فيما عدا وزن 100 حبة، ظهرأتجاة مماثل لمحصول الفول البلدي ومكوناته إلا أن مقدار الأستجابة كان متغير بين المعاملات المدروسة. توضح النتائج أن التأثير النشط

للمكمور مخلفات المزرعة بالنسبة لسماد نترات النشادر كان مصحوبة بزيادات قدرت بنسبة 8.5 ،17.4، 5.5 % لكل من الوزن الجاف ومحصول الحبوب ونسبة البروتين في الحبوب علي التوالي. بينما كان هناك أرتفاع لمعاملات الخلط نتيجة التلقيح البكتيري بمتوسط قدرة 8.7 ، 28.3 ، 15.4 % علي التوالي. فيما عدا محصول القش فقد بينت النتائج أن هناك أختلاف معنوي فى محصول الحبوب الجافة مع أختلاف درجة التباين يرجع هذا أساسا لمصدر النيتروجين المضاف والتلقح بالبكتريا و التداخل بينهم. أما بالنسب لمحتوي القرون و النبات الكامل من النيتروجين وتراكمة فقد ظهر بنفس الأتجاة السابق. بناء على نتائج التحليل الإحصائى لتداخل المعاملات التجريبية أن إستخدام مكمور مخلفات المزرعة كان الأمثل فى زيادة أنتاجية

المحصول ونسبة بروتين حبوب عند أضافتة بمعدل 7 طن للفدان (30 كجم نيتروجين للفدان) لحبوب الفول البلدي وتلقيحها بكتريا الريزوبيم والنامية تحت ظروف الأراضى الجيرية. بالإضافة إلى ذلك ثبت أن إضافة مكمور المزرعة عمل على خفض محتوي التربة من النيتروجين الموجود علي صورة نترات وكذلك رقم حموضة التربة فى نهاية التجربة بالمقارنة بسماد نترات النشادر.