Sustainable use of mineral and biofertilizers in the production of faba bean (*Vicia faba*) grown on calcareous soil.

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ABSTRACT

To evaluate the effect of minimizing N, P and K fertilization levels in the presence of biofertilizers, two field experiments were conducted during the two successive winter season of 2003/04 and 2004/05 at the irrigated research experimental farm of Nubaria. The experimental design was a randomized complete blocks design (RCBD). Main plots consisted of three N, P and K combination levels (low= L, medium= M, and high=H). Meanwhile, the sub-plots consisted of four biological fertilization treatments: control (without inoculation) inoculation with Rizobia (R), infection with Arbuscular Mycorrhiza (AM) and co-inoculation and infection (R+AM).

The results showed that plant height, GP (green pod) yield, and seed yield (SY) of fababean were responded significantly to the mineral fertilization. Inoculation with microorganisms increased biological yield (BY), SY, and staw yield (StY) of faba bean in the two growing season. Nitrogen use efficiency (NUE), phosphorous use efficiency (PUE), and potassium use efficiency (KUE) increased with decreasing fertilizer rate. With high fertilizer level, the coupled inoculation (R+AM) recorded the highest NUE, PUE, and KUE. Soil NH_4^+ concentration increased significantly with the coupled inoculation (R+AM) while it decreased significantly with (R) and (AM) compared to un-inoculated. In contrary, Soil NO_3^- increased significantly with (R), (AM), and (R+AM).

Nutrients uptake by faba bean plants increased significantly with different levels of mineral fertilizers. The coupled inoculation had remarkable influences on all nutrients uptake than single biofertilizers. Concerning, the interaction of mineral and biofertilizers, it was noticed that (R) significantly increased N uptake in straw with L and M NPK. Phosphorous uptake was increased significantly in both straw and seeds with (AM) inoculation at all NPK fertilizer levels. For the coupled inoculation it was noticed that (R+AM) increased significantly N uptake in straw at the three fertilizer levels. Nitrogen, P and K uptake in the whole plants was also significantly increased by the interaction between mineral and biofertilizers. The combined treatment (high NPK+ coupled inoculation) produced the highest P and K in plants, while, the L-NPK with coupled inoculation recorded the highest N uptake for the whole plants.

INTRODUCTION

One of the major concerns, in today's world, is the contamination of agricultural soil. The extensive use of chemical fertilizers and pesticides has caused tremendous harm to the environment. An answer to this is the biofertilizer, an environmentally friendly fertilizer now used in most countries.

In recent years, concepts of integrated plant nutrient management (IPNM) have been developed, which emphasize maintaining and increasing soil fertility by optimizing all possible sources (organic and inorganic) of plant nutrients required for crop growth and quality. This is done in an integrated manner appropriate to each cropping system and farming situation. Improvement in agricultural sustainability requires optimal use and management of soil fertility and soil physical properties, both of which rely on soil biological processes and soil biodiversity. In this context, the long-lasting challenges in soil microbiology are development of effective methods to know the types of microorganisms, and to determine functions which the microbes perform in situ (Tilak, 2005).

Bio-fertilizers are organisms that improve soil fertility and enhance nutrients and water uptake in deficient soils, thereby aiding in better establishment of plants. It also secretes growth substances and antifungal chemicals, as well as improves seed germination and root growth. Moreover, biofertilizers will help for solving such problems as increased salinity of the soil and chemical run-offs from the agricultural fields.

Nitrogen fixation by leguminous crops is a relatively low-cost alternative to N fertilizer for small-holder farmers in developing countries. It can also maintain soil fertility and can be of benefit to the successive crop. Legumes have considerable potential in crop rotations in sustainable agricultural systems in maintaining soil fertility and thus reducing the need for nitrogen fertilizer. Reduced use of nitrogen fertilizer can decrease nitrate leaching from soils and so can reduce eutrophication, which is a major environmental problem worldwide (Amanuel *et al*. 2000).

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Phosphorus is one of the major plant nutrients limit-unavailable to plants (Rodriguez and Fraga, 1999). Most agricultural soils contain large reserves of P, a considerable part of which has accumulated as a consequence of regular application of chemical fertilizers. However, a large proportion of inorganic phosphate added to soil is rapidly fixed as insoluble forms soon after application and becomes unavailable to plants. The superphosphate fertilizer, which contains about 15% of P_2O_5, normally loses its available P proportion when it comes in contact with soil minerals containing calcium carbonates (Lindsay, 1979)

A substantial number of bacterial species, mostly those associated with the plant Rizosphere, may exert a beneficial effect upon plant growth (Glick, 1995). This group of bacteria has been termed “plant growth promoting Rizobacteria” or PGPR (Igual, et al. 2001) and, among them, some phosphate-solubilizing bacteria (PSB) are already used as commercial biofertilizers for agricultural improvement (Subba, 1993). Therefore, for agronomic utility, inoculation of plants by target microorganisms at a much higher concentration than those normally found in soil is necessary to take advantage of their beneficial properties for plant yield enhancement.

Arbuscular mycorrhizal (AM) fungi positively affect the acquisition of mineral nutrients by the host plant by functionally increasing the absorptive surface of the root system. This has been demonstrated in case of phosphorus with numerous combinations of plant and AM fungal species (Smith and Read, 1997). It has also been shown that external AM hyphae are able to take up nitrogen both as NH_4^+ (Frey and Schiepp, 1993) and NO_3^- (Tobar et al, 1994) and translocate N to the host plant in considerable amount. Barea et al. (1989) found that external AM hyphae may utilize N sources that are not available to the roots alone.

An alternative approach for the use of phosphate-solubilizing bacteria, as microbial inoculants, is the use of mixed cultures or co-inoculation with other microorganisms. In this regard, studies on the isolation and selection of microorganisms with ability to promote higher solubilization of phosphorous have been carried out in many works, especially because of the possibility of interaction with microorganisms involved in biological nitrogen fixation (Igual et al., 2001). El-Serafei et al. (2006) reported that there was a slight additional increase in grain and straw yields of wheat when a biofertilizer was applied along with N fertilizer. The N fixing bacteria + P solublizing bacteria combination increased these parameters over the N-fertilizer control. The effect of the N biofertilizer in increasing grain yields was equivalent to N fertilizer application rate of about 13 kg N ha^{-1}.

This study was carried out to evaluate the effect of minimizing N, P and K fertilization levels in the presence of biofertilizers including interaction of N_2 fixing bacteria (R) and P solubility enhancing fungi (AM) with three different levels of mineral fertilizer (L, M, and H) in a soil with low level of available N and P, on Faba bean (*Vicia faba*, L.) yields, plant characteristics, total N, P and K in plant at harvesting time, the response of mineral N, P and K availability in soil will be evaluated.

MATERIALS AND METHODS

Two field experiments were conducted during the two successive winter season of 2003/04 and 2004/05.

1-Experimental site

This study was conducted at the irrigated research experimental farm of Nubaria Agriculture Research Station, North Tahrer, west Nubaria region under the conditions of newly reclaimed calcareous soils irrigated by the surface irrigation system. The used soil is sandy clay loam, has medium permeability while it was well drained. The physical and chemical characteristics of the surface soil (0-20 cm) and sub-surface soil (20-40) were determined according to Page *et al.* (1982) and Klute (1986) and were illustrated in Table (1).

Table 1. The physical and chemical characteristics of the surface and sub-surface soil

Characteristics	surface soil	sub-surface soil
pH	8.2	8.15
EC dS m^{-1}	1.3	1.42
% $CaCO_3$	25.3	21.4
% O.M	0.33	0.36
KCl extractable NO_3 mg kg^{-1}	23.50	29.20
$NaHCO_3$ extractable P mg kg^{-1}	12.10	6.55
NH_4OAC extractable K mg kg^{-1}	380.0	320.50

2-Materials used

Seeds of Faba Bean *Vicia faba* variety Giza blanka were sown. Ammonium nitrate (33.5% N), single superphosphate (15.5% P_2O_5) and potassium sulfate (48% K_2O) fertilizers were used as sources of N, P and K. Two strains of biofertilizers were inoculated to faba bean seeds. *Rizobium meleloti* (R) strain was inoculated to seeds as good root-nodule bacteria for N_2 fixation, whereas Arbuscular Mycorrhiza (AM) inocula spores (mixture of *Glomus macrocarpium*, infected root fragments and mycelium) were used to infect seeds of faba bean as appropriate strains.

3-Experimental Design

The experiment layout was a randomized complete blocks design (RCBD) with four replicates distributed randomly. Main plots consisted of three N, P, and K combination levels (low, medium and high). Meanwhile, the sub-plots consisted of four biological fertilization treatments; control (without inoculation) inoculation with (R), infection with (AM) and finally dual inoculation and infection with (R+AM).

4-Field Experiments

The area of experimental plot was 18 m^2 (6m x 3m), each sub-plot contains 10 rows with 3 m length. The fertilizers: N, P and K were applied with (L), (M) and (H) rates. Nitrogen was fertilized with the rates of 20, 40 and 60 kgN fed^{-1}, P with the rates of 15, 22.5 and 30 kg P_2O_5 fed^{-1} and K with rates of 24, 36 and 48 kg K_2O fed^{-1}. P and K fertilizers were incorporated during land preparation in the soil surface for all plots according two fertilization treatments. Treatments included two factors: (i) three mineral NPK fertilization levels consisted of 20-15-24, 40-22.5-36 and 60-30-48, and (ii) four inoculation and infestation treatments with R and AM included: un-inoculated (control), inoculated with R, infested with AM and coupled inoculation and infestation with R + AM. Seeds of Faba bean in the two experiments were sown in the first week of November 2003 and 2004 during the two growing seasons. All plots received 2 equal applications of N fertilizer according to N fertilization level as NH_4NO_3.

Plant samples were taken 75-80 days after planting to determine (GP). Faba bean plant characteristics, (BY), (SY) and (StY) and yield components were measured 125 days from planting.

Samples of green pods, seeds and straw at harvesting time were dried in a forced-air dryer at 65-70°C. After grinding and wet digested using concentrated sulfuric acid and hydrogen peroxide (FAO, 1980) N, P and K were determined according to Westerman (1988). Finally concentrations and uptake of N, P and K were calculated.

Nitrogen, phosphorus and potassium use efficiencies (NUE, PUE and KUE) were calculated according to formula of Huggins and Pan (1993): N-, P-, K-UE= seed yield $fed^{-1} \div$ added nutrient fertilizer kg fed^{-1}.

5-Statistical Analysis

Data of the present study were statistically analyzed using MSTAT-C software (Freed, 1988). The comparisons among means of the different treatments were carried out; using the Duncan's multiple range tests as illustrated by Gomez and Gomez (1983).

RERSULTS AND DISCUSSION

The used experimental soil is calcareous sandy clay loam with low content of both organic matter and available NPK indicating poor fertility status (Table 1).

1- Effect of different treatments on faba bean biomass

Table (2) showed that plant height, green yield (GP), and seed yield (SY) of fababean were responded significantly to the mineral fertilization. Plant height was increased by about 3 and 2% with the treatment of Med. NPK, and high NPK, respectively. Whereas, High NPK, and Med. NPK increased the (GP) yield by 94 and 31%, respectively. Indeed, the (SY) was increased by 8 and 18% with M and H-NPK, respectively.

The un-inoculated (control) showed the lowest growth rate of all inoculation treatments with yield characteristics Table (2). Inoculation with microorganisms increased BY, SY, and StY of faba bean in the two growing seasons. Significant increase in faba bean yields (BY, SY, and StY) with (R) reached to 3, 1, and 5%, respectively, whereas, (AM) increased the same component by 1, 7, and 2%, respectively. It is obvious to mention that (SY) showed remarkable increase with (AM) treatment.

In case of coupled inoculation and infestation with (R+AM), a significant increase was recorded and reached 14, 19, and 12% for BioY, SY, and StY, respectively.

The results of interaction indicated that faba bean productivity increased insignificantly over control treatments except for seed index which increased significantly. The maximum seed index were recorded with M-NPK+(AM)

For L. fertility treatments, the present results indicated that faba bean responded negatively to inoculation. This may be due to competition for nutrients between plants and different types of microorganisms. The same trend was observed by Gavito and Varela, (1995).

2-Nutrients use efficiency

Nitrogen, phosphorous and potassium use efficiencies (NUE, PUE, and KUE) are presented in Figs 1, 2, and 3. It is noted that NUE, PUE, and KUE increased with decreasing fertilizer rates. This increasing may be due to the beneficial and enhancement of microorganisms in supplying fababean with nutrients.

In case of high fertilizer level, the coupled inoculation (R +AM) recorded the highest NUE, PUE, and KUE (27.5, 54.9, and 34.3). However, the treatment of (R) reached 26.2, 52.5, and 32.8, and the treatment of

In contrary, with M and L fertilizer rate the (AM) treatment recorded higher increasing than (R) treatment. This increase could be attributed to the effectiveness of (AM) hyphae to take up N and K as well as P (Johansen,

1999). The same trend was observed by Awad et al. (2004) (AM) reached 25.3, 50.6, and 31.6 for NUE, PUE, and KUE, respectively.

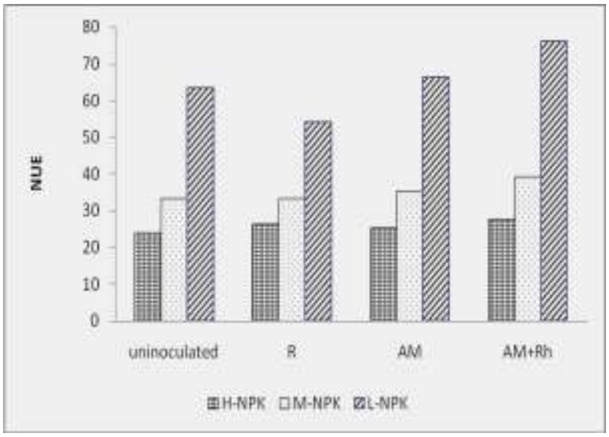

Fig.1 Effect of inoculation with (R), (AM), and (AM+R) on NUE under three mineral fertilizer levels.

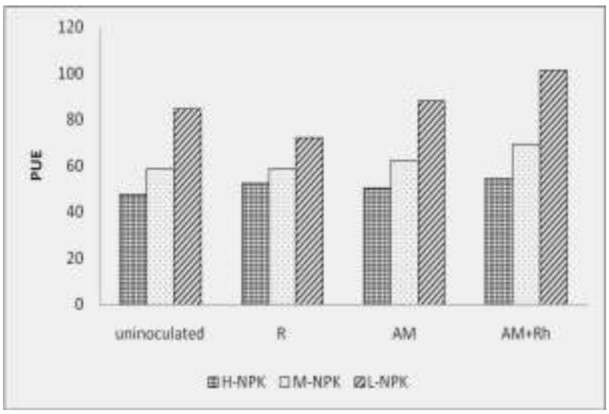

Fig.2 Effect of inoculation with (R), (AM), and (AM+R) on PUE under three mineral fertilizer levels.

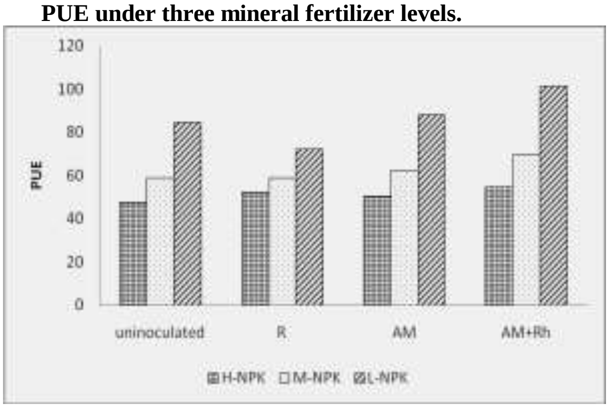

Fig.3 Effect of inoculation with (R), (AM), and (AM+R) on KUE under three mineral fertilizer levels.

3-Residual soil nutrients

Regarding the effect of mineral fertilization, data in Table (3) showed that NH_4^+ concentration in soil was insignificantly affected by the level of N fertilizer applied, while NO_3 was increased significantly with increasing N fertilizer level. This increase reached 12% at M-NPK. The same trend was observed with available P and exchangeable K. The increase in soil nutrients means that the fertilizers applied were over plant requirement.

Concerning, the effect of biological fertilization, data in Table (3) showed that soil NH_4^+ concentration increased significantly by 2% with the coupled inoculation (R+AM) while it decreased significantly by 0.4 and 8% with (R) and (AM) compared to un-inoculated, respectively. In contrary, NO_3^- increased significantly with (R), (AM), and (R+AM). This may be due to that external hyphae and ryzobium seem to prefer NH_4^+ to NO_3^-. Soil available P and exchangeable K had the same trend as soil NO_3^-. The depletion of soil NH_4 concentration induced by the presence of (R) or (AM) is due to the parallel utilization and transport of NH_4^+ by the external hyphae or ryzobium. This may indicate that they absorbed a considerable NH_4, and this demonstrates the decreasing in plant height with (R) and (AM) as shown in Table (2). Evidence has been given for (R) and (AM) fungal utilization of NH_4 (Johansen, 1999). In case of coupled inoculation, this depletion was not observed.

Table (3) illustrated also the interaction between different levels of NPK and the biological fertilizers inoculation. It is clear that (R) depleted soil NH_4^+ at L-NPK level due to the competition between plant uptake and (R) acquisition, and at H-NPK level it decreased. This may be due to the increasing in tillers number Table (2). However at M-NPK level soil NH_4^+ increased.

The (R) induced an increased about 21% relative to the un-inoculated at the H-NPK while the increasing was about 6% only at M-NPK level. This increase may be due to the growth substances secreted by microorganisms which solubilize nutrients in the rhizosphere.

On the other hand, (AM) caused depletion in soil NH_4^+ at the three levels of mineral fertilizers compared with the un-inoculated treatment. The same trend was observed with the coupled inoculation (R+AM) except at L-NPK level, Soil NH_4^+ increased by 8% relative to the un-inoculated. This is may be due to the decreasing in plant height (Table2) which reflects the decreasing of N uptake by plants.

In contrary, Soil NO_3 increased with increasing NPK level and this confirmed the previous discussion that (R)

Table3. The amount of residual soil mineral N, available P (Av.P) and Exchangeable K (Exch.K) as affected by levels of N, P, and K fertilization and biological fertilizers inoculation.

Treatment		Mineral N		Av. P	Exch. K
Min. F	Bio. F	NH_4	NO_3		
			µg g^{-1}		
L- NPK		29.11	32.73	22.05	313.33
M-NPK		28.79	36.93	24.92	374.58
H-NPK		29.11	36.30	25.93	387.08
	LSD0.05	ns	3.36	0.93	47.52
	Un-inoculated	29.50	32.50	21.99	337.22
	Rhizobia (R)	29.36	36.50	23.01	351.67
	Mycorrhiza (AM)	27.03	35.50	25.23	356.11
	R+ AM	30.14	36.44	26.90	388.33
	LSD0.05	1.80	2.13	1.31	12.06
	Un-inoculated	28.49	29.05	19.93	280.00
L- NPK	Rhizobia (R)	28.13	33.08	21.77	315.00
	Mycorrhiza (AM)	28.93	31.62	22.85	323.33
	R+ AM	30.90	37.17	23.66	335.00
	Un-inoculated	29.48	35.82	22.59	365.00
M-NPK	Rhizobia (R)	30.80	38.87	23.18	353.33
	Mycorrhiza (AM)	25.47	35.53	26.07	376.67
	R+ AM	29.42	37.50	27.85	403.33
	Un-inoculated	30.52	32.57	23.44	366.67
H-NPK	Rhizobia (R)	29.15	38.60	24.30	386.67
	Mycorrhiza (AM)	26.69	39.35	26.77	368.33
	R+AM	30.10	34.67	29.20	426.67
	LSD0.05	3.12	3.69	2.27	20.90

Min F: mineral NPK fertilizers level Bio.F: biological fertilizers inoculation

ns : statistically not significant

preferred NH_4^+ than NO_3^-. The increase in soil NO_3^- concentration with (AM) inoculation was less than with (R), this nay be due to the (AM) can absorb NO_3 as well as NH_4^+.

Available P increased by 9, 2 and 3% with L, M, and H-NPK level, respectively in plant inoculated with (R) compared by un-inoculated. The highest soil P concentration recoded at L-NPK level. This may be related to the lower plant uptake at this level.

The same trend was observed with (AM) which was higher than (R). This was attributed to the effectiveness of (AM) to solubilize P.

Exchangeable K also had the same trend as available P with NPK levels. This means that using of biofertilizer could improve soil fertility.

4-Nutrient uptake

Nitrogen, P, and K uptake by faba bean plants increased significantly with different levels of mineral fertilizers (Table4). The highest value of NPK was noticed when plants fertilized with H- NPK fertilizers except for N uptake in straw where the highest value was obtained with M-NPK. This may be due to that heavy N fertilizer applications reduced yield as well as N uptake. These results matched well with those of Barakat and Gabr, (1998) who found that tomato fruit yield usually increased with moderate N dressings.

Regarding the effect of biological fertilizers, it was noticed in Table (4) that (R) increased significantly N, P, and K uptake in straw, seeds and total plants except K uptake in straw which decreased in rhizobia treated plants. This may be referred to the dilution effect, as the plant dry matter increased due to (R) inoculation. On the other hand, (AM) significantly increased NPK uptake in straw, seeds and total plants, except for N uptake in straw. This could be due to the effect of (AM) inoculation in solubilizing P and increased root surface area to take up different nutrient efficiently. Similar results were obtained by Koreish et al., 2004. Using coupled inoculation (R+AM) significantly resulted in highest N, P, and K uptake in straw, seeds, and total plant compared with the un-inoculated plants. The coupled inoculation had remarkable influences on all traits than single biofertilizers. The improving effects of

biofertilizers on N, P, and K uptake can be related to the role of N_2-fixing bacteria as well as (AM) in increasing nutrient uptake. Moreover, the hormonal exudates of the biofertilizers bacteria can modify root growth, morphology, and physiology, resulting in more absorption of nutrient.

Concerning, the interaction of mineral and biofertilizers, it is noticed in Table (4) that (R) significantly increased N uptake in straw with L and M-NPK by 16 and 11% relative to un-inoculated, while, it decreased by 2.5% at high NPK. This may be due to the decrease in (R) activity with H-NPK application. In the same time, P uptake was significantly increased by 10, 8, and 2.5% relative to un-inoculated. In contrary, (R) significantly decreased K uptake in straw with the three N, P, and K fertilizer levels. Meanwhile, it increased significantly K uptake in seeds by 9 and 13% relative to un-inoculated plants with L and M-NPK, respectively.

Phosphorous-uptake was increased significantly in both straw and seeds with (AM) inoculation at all N, P, and K fertilizer levels. The observed increase in seeds was 36, 29, and 26% relative to un-inoculated plants with the three fertilizer levels, L, M, and H, respectively. While, the P-uptake observed in straw was lower (42, 15, and 5%) relative to un-inoculated at the three fertilizer levels, respectively. This could be to that plants stored P in seeds.

For the coupled inoculation, it was noticed in Table (4) that (R+AM) increased significantly N uptake in straw by 35, 15, and 11% relative to un-inoculated plants at the three fertilizer levels, respectively; and this confirm the previous results for increasing NUE at lower N fertilizer applications. The P uptake was increased significantly in seeds than in straw with coupled inoculation compared to un-inoculated except for M-NPK. Also, coupled inoculation increased significantly K uptake in both straw and seeds with all fertilizers levels.

Nitrogen, P, and K uptake in the whole plants was also significantly increased by the interaction between mineral and biofertilizers. The combined treatment (high NPK+ coupled inoculation) produced the highest P and K in whole plants. While, the L-NPK with coupled inoculation recorded the highest N uptake for the whole plants.

Finally, it could be concluded that biofertilizers improve soil fertility and enhance nutrients uptake in deficient soil, thereby aiding in better establishment of plants. Thus, biofertilizers are important if we are ensure a healthy future for the generations to come.

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الملخص العربي

الأستخدام المستدام للأسمدة المعدنية والحيوية فى انتاجية نبات الفول النامى فى الأرض الجيرية.

فاطمة كمال شريف ، مُحَّد السيد مرسى، وأحمد عوض

لدراسة تأثير إستخدام بعض الأسمدة الحيوية على التقليل من معدل إضافات الأسمدة المعدنية فقد أقيمت تجربتين فى موسمين متتتالين 2004/2003 2005/2004 فى منطقة النوبارية وقد نفذت التجارب باستخدام تصميم القطاعات كاملة العشوائية RCBD .

تضمنت المعاملات ثلاث معادلات من الأسمدة المعدنية N, P and K مع تلقيح نبات الفول بالريزوبيا أو الميكوريلزا وكذلك الإثنين معاً. وقد أظهرت النتائج أن طول النبات والمحصول الأخضر وأنتاج الحبوب قد إستجاب معنوياً للأسمدة المعدنية. وكذلك إستخدام الأسمدة الحيوية أدت إلى زيادة BY,SY,STY خلال الموسمين.

كذلك وجد أن كفائة استخدام الأسمدة المعدنية KUE,PUE,NUE ازدادت بإنخفاض معدلات الأسمده المعدنيه. عند المستوى المرتفع من الأسمده المعدنيه فإن التلقيح المختلط سجل أعلى كفائة للأسمدة المعدنية المستخدمه. كما وجد أن الأمونيوم فى الأرض بعد الحصاد قد ازداد تركيزه معنويا فى حالة الأسمدة الحيوية

المختلطة، بينما انخفضت فى الأسمدة الحيوية المنفردة. وعلى العكس فقد ازداد تركيز النترات فى جميع الأحوال مما يدل على أن الميكوريزا تفضل الأمونيوم عن النترات. وقد أخذ الفوسفور المتاح والبوتاسيوم نفس الاتجاه كما فى حاله النترات.

كذلك وجد أن لمتصاص العناصر المختلفة NPK فقد إزداد معنوياً بزيادة الأسمده المعدنيه المضافة.

كما أن الأسمدة الحيوية المختلطة سجلت تأثيراً ملحوظاً على العناصر المتمصه NPK بواسطة النبات أفضل من السماد الحيوى المنفرد. أما بالنسبة للتأثير المتداخل بين الأسمده المعدنيه والحيوية فقد وجد أن الرينزوبيا أدت إلى زيادة N الممتص بواسطة الفول فى القش عند المستوى المنخفض والمتوسط بينما إزداد إمتصاص P مع الميكوزيزا عند كل معدلات التسميد المعدني. كذلك لوحظ إزدياد امتصاص N فى القش عند كل المستويات التسميد المعدنى والتسميد الحيوى المختلط.

وبحساب إمتصاص NPK فى النبات الكامل وجد أنه إزداد مع التسميد عند مستويات التسميد المعدنى المختلفة.