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Effect of Endomycorrhizal Fungi, N_2- Fixing Bacteria and Biological Potassium Fertilizer on the Yield and Quality of *Nigella sativa* in Calcareous Soil

Amal K. Abou El-Goud[*], Amal M. Aboul-Nasr[*], M.E. El-Fayoumy[**] and E. Koreish[***]

[*]*Agricultural Botany Department, Agricultural Microbiology, Faculty of Agricultural, Saba-Basha, Alexandria, University,* [**]*Soil, Water and Environment Research Institute, Nubaria Research Station, Agricultural Research Centre, Cairo and* [***]*Soil Microbiology Department, Faculty of Agricultural, Alexandria University, Alexandria, Egypt.*

TWO FIELD experiments were carried out during winter seasons 2008/2009 and 2009/2010 at the Farm of Nubaria Agricultural Research Station. The aim of the work was to study the effect of the mycorrhizal fungus *Glomus etunicatum*, non-symbiotic N_2-fixing bacteria (Halex), and *Bacillus circulans*, under different rates of mineral N, P, and K fertilizers (0, 25, 50, 75 and 100%) on the yield and quality of *Nigella sativa* plants growing in calcareous soil. The experiments were arranged according to a completely randomized split–plot block design with four replicates. The highest percentage of mycorrhizal root length colonization were 38.3% and 54.2% in case of plants inoculated with *G. etunicatum* in the presence of $P_{75\%}$ and the mixture of inocula at the rate of $NPK_{75\ \%}$ of mineral fertilizers, respectively, whereas the un-inoculated plants ranged (18.4%). The highest percentage increase in NPK- uptake of shoots in case of inoculated *Nigella* plants with a mixture of inocula under the different rates of mineral NPK fertilizers compared to un-inoculated once. Significant increase was obtained in seed yield (534.4 kg/fed) in case of plants inoculated with a mixture of inocula (Halex, *G. etunicatum* and *B. circulans*), as compared to the un-inoculated plants (516.0 kg/fed) in the presence of $NPK_{75\%}$ of the recommended dose of NPK fertilizers. The highest significant value of fixed oil percentage was (34.7%) by inoculation mixture of inocula compared to un-inoculated once (33.0%) in the presence of $NPK_{75\%}$ of the recommended dose of NPK-fertilizers. From the above mentioned results, it is clear the positive use of beneficial soil microorganisms on the plant growth and yield, in order to decrease using mineral fertilizers and chemical pollution.

Keyword: AM fungi, Non symbiotic N_2- fixing bacteria (Halex), Biological Potassium Fertilizer (BPF), Calcareous soil, *Nigella* seed yield and Fixed oil %.

Black cumin seeds from *Nigella sativa* are normally used for medicinal purpose, as they contain 21% protein, 35% carbohydrates, 36% fat, and 15 kinds of amino acids, including 9 essential amino acids (Khalifah, 1995 and Ara Der Marderosian, 2001). *Nigella* seeds play an important role in recovering humans from many diseases and for their anti-asmatic activity. Pharmacological activity of oil from *Nigella sativa* is due to the occurrence of two substances, thymoquinon and nigellon (Mahfous and El- Dakhakhny, 1960). The cultivated area of *N.sativa* in Egypt cover about 122 feddans yearly and the seed production reach 53 tons per year (Abaza, 2001).

Calcareous soil occupy wide areas in Egypt. This soil suffers from high of $CaCO_3$ content high soil pH, normally basic in reaction and low activity of beneficial soil microorganisms (El-Fayoumy *et al*., 2000, Entry *et al*., 2002 and Mahrous *et al*., 2006). Under these conditions, the availability of macro- and micro-nutrients is not enough for a good growth and production. In order to increase the productivity, huge amounts of mineral NPK fertilizers are added in these soil, thus leading to significant environmental pollutions (Hegazi *et al*., 2003).

Halex contains some microbial species, *i.e. Azotobacter, Azospirillum* and *Klebsiella*, which are the main nitrogen fixers occurring in calcareous soils. These bacteria utilize few nitrogenous compounds, N_2 and ammonium, to increase both soil fertility and the population level of soil beneficial microorganisms, as well as to accelerate microbial processes in the rhizosphere of inoculated plants (Hassouna and Hassanein, 1996). They also increase the amounts of fixed N in the plants and the amount of N left in the soil. Such microorganisms are useful for recycling elements, preserve natural resources and protecting against the deleterious effects of chemical pollution (Alaa El-Din *et al*., 1982).

Arbuscular mycorrhizal (AM) fungi play a critical role in nutrient cycling, they increase the root surface area, and alleviate the effects of adverse soil pH, thus favouring the regular ecosystem functions (Grant *et al*., 2005). Mycorrhizal associations enhance nutrient uptake, improve the plant growth, and modify the soil fertility, yield production and (Helgason and Fitter, 2005). AM fungi stimulate synergistic relationship with other soil beneficial microorganisms, act as biocontrol agents, preventing pathogens infection and enhancing the plant growth and production (Ortas *et al*., 2001).

Furthermore, the enhancing effect of Biological potassium fertilizer (BPF) on the host plant was due to the increase of K-uptake, along with N and P, from the soil (Berge *et al*.,1990). The additive effects of increased K mobilization and N_2 – fixation on the availability of K, N and consequently P, along with the biologically active substances, on the enhancement of root development will evidently promote assimilation of all the elements of mineral nutrition, thus increasing the growth and yield of the host (Balable, 1997).

The objectives of this investigation were to study the positive effect of AM fungi, non-symbiotic N_2-fixing bacteria (Halex), and the biological potassium fertilizer (BPF) under different rates of mineral N, P and K fertilizers (0%, 25%, 50%, 75%, and 100%) on the growth, yield, and quality of *Nigella sativa* plants growing in calcareous soil. Moreover, the interaction between AM fungi, Halex and BPF for achieving the suitable quantity of mineral NPK fertilizers was investigated, in order to decrease the use of mineral fertilizers and the chemical pollution.

Materials and Methods

Two field experiments were carried out during two winter seasons (2008/09 and 2009/10 at the Farm of Nubaria, Agricultural Research Station.

The field experiments were arranged according to a split-plot design with completely randomized blocks, containing four replicates. Each experimental unit (plot) consisted of four rows, 3m long and 3.5m wide, with 20 cm distance between each hill. The total area of each unit was 10.5 m^2 and it contained 60 plant .

Black cumin seed *Nigella sativa* was provided by the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Halex, containing non-symbiotic N_2 -fixing bacteria of three genera, *Azotobacter*, *Azospirillium* and *Klebsiella*, was provided by the Biofertilizer Unit, Plant Pathology Department, Faculty of Agriculture, Alexandria University. Halex was used at the rate of 10 g/kg seed and it was applied as seed dressing.

Mycorrhizal strain of *Glomus etunicatum,* which was isolated from the Experimental Station of Alexandria University at Abies, Egypt (Aboul–Nasr, 1993) was used. The inoculum consists of expanded clay aggregates (2-4 mm in diameter), containing chlamydospores and mycelium of the fungus (Aboul – Nasr, 2004). The inoculum was applied prior to sowing at the rate of 5.0 g/hill. The control plants received the same amount of heat sterilized expanded clay.

BPF was provided by the the Microbiology Institute, Hebei Academy of Sciences, China. *Bacillus circulans* strain was produced at the rate of 10^{-2} -10^{-6} concentrations, serial dilutions technique was used. *Nigella* seeds were dipped in the above bacterial suspension concentrations for two hours before sowing and 25 ml from this suspension was added after sowing on each hill.

Nitrogen fertilizer (ammonium-nitrate, 33.5% N) was used at the rates of 0, 25, 50, 75, and 100% of the recommended dose. The recommended dose was 200 kg/fed, and it was added at three equal doses, 21, 45, and 80 days after sowing. Phosphorus fertilizer (Mono-calcium phosphate, 15.5% P_2O_5) was used at the rates of 0, 25, 50, 75, and 100 % of the recommended dose. The recommended dose was 300 kg/fed, and it was added at the time of preparation the soil at one dose. Potassium fertilizer (Potassium sulphate, 48% K_2O) was applied at rates of 0, 25, 50, 75, and 100 % of the recommended dose. The recommended dose was 150 kg/fed, it was added at the second dose of nitrogen fertilizer.

TABLE 1. Treatments as applied in two winter seasons (2008/09 and 2009/10).

Bio-fertilizers		Mineral NPK Fertilizers
Halex (*Atotobacter, Atospimillum* and *Klebsiella*)	–* +**	N_0, N_{25}, N_{50}, N_{75}, and $N_{100\%}$ of the recommended dose + $P_{100\%}$ + $K_{100\%}$
AM fungi (*Glomus etunicatum*)	– +	P_0, P_{25}, P_{50}, P_{75}, and $P_{100\%}$ of the recommended dose + $N_{100\%}$ + $K_{100\%}$
BPF (*Bacillus circulans*)	– +	K_0, K_{25}, K_{50}, K_{75}, and $K_{100\%}$ of the recommended dose + $N_{100\%}$ + $P_{100\%}$
The mixture of inocula (Halex, AM fungi, and BPF)	– +	NPK_0, NPK_{25}, NPK_{50}, NPK_{75}, and $NPK_{100\%}$ of the recommended dose

* (-) Un-inoculated (control) plants
** (+) Inoculated plants

Physicochemical characteristics of the soil (0-30 cm depth) of the experimental field were as follows: pH= 8.3, $CaCO_3$ % = 22.5, Organic Matter (OM) = 4.0%, availablemacro-nutrients N= 48.3 ppm, P= 4.0 ppm, K= 115.5 ppm. Soil texture was sandy clay-loam (Page *et al*., 1982, Klute, 1986). The percentage of mycorrhizal root length colonization was stained according to Koske and Gemma, 1989. The percentage of AM root colonization was determined 9 weeks after sowing and estimated according to (Giovannetti and Mosse, 1980). At the harvest, shoot samples were collected and dried for measuring the percentage of phosphorous and potassium according to Jackson (1958) by using the vanadomolybdate yellow method and the flam photometer, respectively. Total nitrogen content in the shoots was determined according to Chapman and Pratt (1961). Determination of the total fixed oil percentage in seeds was done by using the BP method, according to Harold *et al*., (1981). Seed yield for each plot was weighted and related to kg/fed.

Data were submitted to the analysis of variance and the mean of the treatments were compared by the LSD test at P≤0, 05 (Snedecor and Cochran, 1981). Percentage of mycorrhizal root length colonization were converted into Bliss angular values before the analysis (Steel and Torrie, 1980).

Results and Discussion

Percentage of mycorrhizal root length colonization

In control plants, the proportion of mycorrhizal root length was 18.4% due to AM fungi naturally occurring in the soil. Plants inoculated with *Glomus etunicatum* under $P_{75\%}$ and $P_{100\%}$ showed a percentage of mycorrhizal root length colonization of 38.3% and 39.2 % ,as an average for the two growing seasons, respectively (Fig. 1a). No significant differences were found between $P_{75\%}$ and $P_{100\%}$ in case of inoculated plants. The highest percentage of mycorrhizal root length colonization was 54.2% in case of plants inoculated with a mixture of inocula (*G. etunicatum*, Halex, and *Bacillus circulans*) compared to un-inoculated control (22.5%) under the rate $NPK_{75\ \%}$ of the recommended dose of mineral NPK fertilizers (Fig. 1b). Sainz *et al*. (1997), and Elwan, (2001) recorded that the extent of root colonization was greatest in inoculated plants compared to un-inoculated once. Abd El-Ghany, (2003) showed that in wheat rhizosphere the highest percentage of mycorrhizal root lengh colonization was obtained on plants treated with dual inocula, *i.e.* AM fungi and *B. megaterium.*

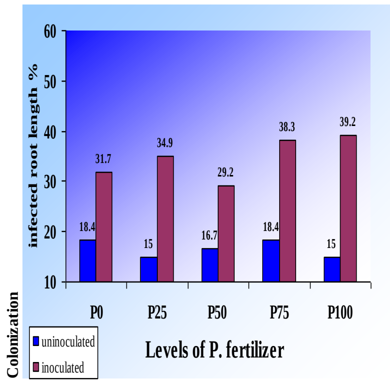

Fig 1a.

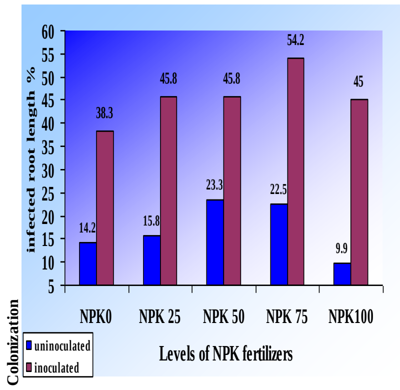

Fig. 1b.

Fig. 1. Effect of treatments with *Glomus etunicatum* alone or with a mixture of bioinocula, on the AM root length colonization of *N. sativa* plants, growing under different rates of mineral N, P, and K fertilizers.

NPK-uptake (kg/fed) in shoots

The percentage increase in case of *Nigella* plants inoculated with Halex (25.5, 23.1, 15.8, and 4.3%) more than the un-inoculated plants, under different rates of mineral nitrogen fertilizer, respectively (Fig. 2a). A significant increase in P and K uptake was observed in case of plants inoculated with Halex under different rates of N- fertilizer during both seasons. The percentage increase of P-uptake were 7.3, 9.6, 8.3, and 4.3% in case of plants inoculated with *G. etunicatum* as compared to un-inoculated plants at the rates of $P_{0\%}$, $P_{25\%}$, $P_{50\%}$, $P_{75\%}$ and $P_{100\%}$, respectively (Fig. 2b). Significant increase in N uptake was reported in case of plants inoculated with AM fungi. During the two seasons, no significant increase in K-uptake was observed by comparing inoculated mycorrhizal and un-inoculated plants, under different rates of P fertilizer (Fig. 2b). The inoculation with *Bacillus circulans* increased the uptake of K and N more than un-inoculated plants (Fig. 2c). A relative percentage increase was noticed in NPK–uptake (kg/fed) in plants inoculated with a mixture of inocula (*G. etunicatum*, Halex, and *Bacillus* *circulans*) more than in the un-inoculated one (Fig. 2d). Generally, the increased percentage of NPK uptake (kg/fed) in *N. sativa* was reduced by the increasing of the amount of mineral NPK fertilizers. Linderman (1992) reported that the extra-radical hyphae of AM fungi can bridge the zone of nutrient depletion adjacent to the root and increase the availability of immobile elements. Aboul-Nasr (1994) cleared that the application of *G. etunicatum* on *Tagtes erecta* and *Zinnia elegans* produced a positive effect on plants, fastening flowering, moreover, the mycorrhizal plants contained significantly higher amounts of P, N, and K in shoots than the un-inoculated plants. Also, Ortas *et al.* (2001), found that inoculation of green pepper with AM fungi lead to increase the uptake of N, P, and K from the soil. Korish *et al.*, (2004) reported that the inoculation of faba bean or wheat with a mixture of bio-fertilizers lead to increase the N, P and K uptake than un-inoculated plants.

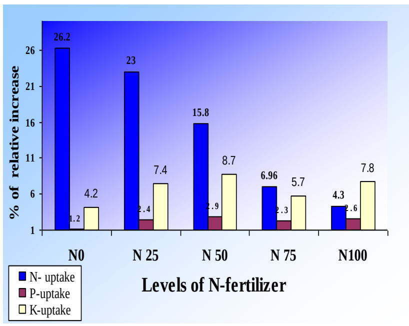

Fig. 2a.

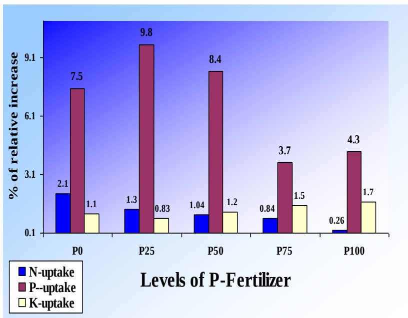

Fig. 2b.

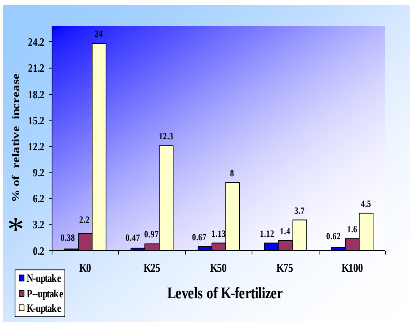

Fig. 2 c.

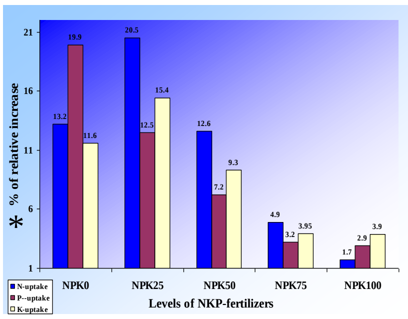

Fig. 2 d.

(*) % of relative increase = % Increase or decrease to un-inoculated (control) plants %

Fig. 2. Effect of inoculaion with G.*etunicatum* alone or with the mixture of inocula under the different rates of mineral N, P, and K fertilizers on NPK – uptake (kg /fed) in shoots of *Nigella sativa*. Data represent the average of two growing seasons.

Seed yield (kg/fed) and fixed oil percentage

The highest seed yield was 538.0 and 534.4 kg/fed, as an average of the two growimg seasons, and they were obtained in case of *Nigella* plants inoculated with Halex alone or with a mixture of inocula (Halex, *G. etunicatum*, and *B. circulans*), as compared to the un-inoculated plants (512.2 and 516.0 kg /fed), under the rates of $N_{75\ \%}$ and $NPK_{75\ \%}$ of mineral NPK fertilizer (Table 2), respectively. No significant differences were found in seed yield (kg/fed) between plants inoculated with Halex and a mixture of inocula, under the rate 75% of mineral N, P and K fertilizers. The percentage increase was 4.9% in case of plants inoculated with *Glomus etunicatum* (505.7 kg/fed), as compared to un-inoculated plants (481.7 kg/fed) at the rate of $P_{75\ \%}$ of the recommended dose of phosphorus fertilizer (Table 2). Significant increase in fixed oil percentage was obtained in case of plants inoculated with either Halex, AM fungi or BPF alone or as a mixture of inocula, more than in the un-inoculated plants under all different rates of mineral N, P and K fertilizer (Table 2). *N. sativa* seed inoculated with Halex, *Glomus etunicatum*, or *Bacillus circulans* alone or as a mixture, lead to an increase of the percentage of fixed oil (32.7, 33.9, 31.2 and 34.7%), compared to un-inoculated plants (31.9 , 33.2 , 30.4 and 33.0 %, respectively) under the different rates (0, 25, 50, 75 and 100%) of the recommended mineral N, P and K fertilizers (Table 2). The highest percentage of relative increase in fixed oil percentage was 5.2, in case of plants inoculated with a mixture of inocula, more than the un-inoculated plants, in both seasons at the rate $NPK_{75\ \%}$ of the recommended dose of mineral NPK fertilizers (Table 2). Mahmoud and Amara (2000) reported that the bio-fertilization under the 50% of NPK dose of mineral fertilizer gave the highest yield of inoculated tomato seeds than un-inoculated one. Yassen, (2002) found that the inoculation of potato plants with a mixture of bio-fertilizers lead to significant increase of total yield, as compared to un-inoculated plants. From the above mentioned results, it is clear a positive increase in the presence of the different type of biocontrol inoculum, especially in the case of the mixture of inocula at the rate 75% of mineral NPK fertilizers, thus allowing a reduction in their use and chemical pollution, and an increase of yield production.

Conclusion

Mixed inoculation of *Nigella sativa* plants using non symbiotic N_2- fixing bacteria (Halex), *Glomus etunicatum* (AMF), and biological potassium fertilizer (*Bacillus circulans*), alone or in combination, determined significant increase in seed yield (534.4 kg/fed) and fixed oil percentage in the presence of 75% of the recommended dose of N, P and K fertilizers. The interaction among AM fungi, non-symbiotic N_2-fixing bacteria and a biological potassium fertilizer (BPF) appear to be essential to promote the plant growth, the NPK uptake in shoots, and seed yield production and quality. From the above mentioned results, it is clear that a positive effect of beneficial microorganisms, leading to a decrease in the amount of mineral N, P and K fertilizers and a reduction of chemical pollution in calcareous soil.

TABLE 2. Effect of inoculation with *G. etunicatum,* Halex, *B. circulans,* alone and as a mixture of inocula, on seed yield (kg/fed) and fixed oil percentage from *N. sativa* plants grown under different rates of mineral N, P, and K fertilizers. Data are the average of two growing seasons (2008/10).

Parameters			Seed yield (Kg\fed)			Fixed Oil %		
Bio-fertilizers / mineral fertilizers			Un- Ino.	Ino.	± %	Un-Ino.	Ino.	± %
Halex	N-fertilizer	0	183.0*	198.8	8.6	29.1	29.9	2.7
		25	317.8	328.8	3.5	30.1	30.7	1.9
		50	466.4	475.7	1.9	31.2	32. 0	2.6
		75	521.2	538. 0	3.2	31.9	32.7	2.5
		100	513.4	538.9	4.9	31.7	32.6	2.8
L.S.D. 0.05			15.708			0 .1953		
Glomus etunicatum	P-fertilizer	0	471.1	478.7	1.6	30.5	31. 0	1.6
		25	474.4	486. 0	2.5	31. 0	31.6	1.9
		50	470.5	491.4	4.4	32. 0	33.1	3.4
		75	481.7	505.7	4.9	33.2	33.9	2.1
		100	481. 0	498.9	3.4	33.6	33.7	0 .3
L.S.D. 0.05			7.757			0 .1948		
Bacillus circulans	K-fertilizer	0	463.1	482.3	4.2	29.3	30.2	3.1
		25	474.3	491.2	3.6	29.9	30.5	2.1
		50	482.2	492.5	2.1	30.2	30.7	1.7
		75	498. 0	505.9	1.6	30.4	31.2	2.6
		100	479.9	499.8	4.2	30.6	31.4	2.6
L.S.D. 0.05			6.5753			0 .1501		
a mixture of inocula	NPK-fertilizer	0	387.5	410.5	5.9	30.4	31.2	2.6
		25	417.6	438. 0	4.9	31.2	31.7	1.6
		50	456.2	478.9	4.9	31.6	32.8	3.8
		75	516.1	534.4	3.6	33. 0	34.7	5.2
		100	511.9	532.4	4.1	33.1	33.9	2.4
L.S.D. 0.05			7.9735			0 .2151		

± % Increase or decrease to un-inoculated (control) plants.
* Mean vales of 4 replicates per each treatment.
Plant age 25 weeks.

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تأثير فطر الميكوريزا الداخليه و الكائنات الدقيقه النافعه علي إنتاج و جودة نباتات حبة البركه في الأراضي الجيريه

امال كرم ابو الجود* ، امال محمد ابو النصر ، محمد عصمت الفيومي** و عصام قريش*****

*قسم النبات والميكروبيولوجية الزراعية – كلية زراعة سابا باشا – جامعة الاسكندرية – **معهد بحوث التربة والمياه والبيئة – محطة بحوث النوبارية – مركز البحوث الزراعية – القاهـــرة و*** كلية الزراعة – جامعة الاسكندرية – الاسكندرية –مصر.

تمت التجربة في المزرعة التجريبه بمحطة البحوث الزراعيه بالنوباريه (الأراضي الجيرية) خلال الموسمين الشتويين ٢٠٠٨/٢٠٠٩ و ٢٠٠٩/٢٠١٠ . بهدف دراسة التأثير الإيجابي لفطر الميكوريزا الداخليه و البكتريا المثبتة للأزوت الجوي لا تكافليا و البكتريا المذيبة للبوتاسيوم المعدني كلا علي حدة أو التأثير الإيجابي لمخلوط الملقحات الحيويه تحت خمس مستويات مختلفة (صفر ، ٢٥ ، ٥٠ ، ٧٥ و ١٠٠٪ من الجرعة الموصي بها) من الأسمدة المعدنيه (النيتروجين – الفوسفور – البوتاسيوم). أوضحت النتائج أن نسبة تواجد فطر الميكوريزا الداخليه في حالة التلقيح بها مختلطه مع مخلوط الملقحات الحيويه السابق ذكرها (٥٤,٢ ٪) عند ٧٥ ٪ من مخلـــوط الأسمدة المعدنيـــة أعلـــي من التلقيح بفطـــر الميكوريـــزا منفردة (٣٨,٣ ٪) عند ٧٥ ٪ من السماد الفوسفاتي المعدني و١٠٠ ٪ من السماد النيتروجين والبوتاسيوم المعدني وأعلي انتاجية من بذور حبة البركه (٥٣٤,٤ كجم/ فدان) في حالة التلقيح بمخلوط الملقحات الحيويه مع ٧٥ ٪ من الجرعة الموصي بها من مخلوط الأسمدة المعدنية (النيتروجين + الفوسفور+ البوتاسيوم)، كما أن نفس المعاملـــة أدت لزيادة معنويـــة في نسبة زيت حبة البركة (٣٤,٧ ٪) و كذا زيادة معنوية في إمتصاص العناصر الكبري من النيتروجين و الفوسفور والبوتاسيوم في نباتات حبة البركه الملقحه مقارنة بالنباتات الغير ملقحه . وهذا دليلا علي التأثير الإيجابي للتداخل بين الكائنات الدقيقة النافعة في تقليل الكميات المستخدمه من الأسمده المعدنية (٢٥ ٪ من كلا من النيتروجين ، الفوسفور والبوتاسيوم) وبالتالي الحد من التلوث الكيميائي للتسميد المعدني مع زيادة الإنتاج من بذور وزيت حبة البركة.