Egypt. J. Microbiol. **38**, No. 1, pp . 75 - 88 (2003)

Assessment the Impact of Certain Growth Promoting Rhizobacteria Strains on Symbiotic Effectiveness of Arbuscular Mycorrhizal Fungi

M. Attia and Nemat M. Awad

Agricultural Microbiology Department, National Research Centre, Dokki, Cairo, Egypt. Email: magdyattia@hotmail.com

A POT experiment was carried out to determine the effect of different combinations of arbuscular mycorrhizal fungi (AMF) and five strains of plant growth rhizobacteria (*Azospirillum, Azotobacter, Enterobacter, Klebsiella, Pseudomonas or Bacillus*) on mycorrhizal formation and growth of wheat and tomato plants in calcareous or alkaline soils. Results indicated that inoculation with GPRB strains and AMF increased root, shoot and total dry weight of tomato plants significantly in both soils compared to inoculation with AMF. Nitrogen and phosphorus uptake were also increased in both soils. No synergistic effect between mycorrhizal fungi and GPRB was observed in mycorrhizal root infection and mycorrhizal spore numbers in tomato and wheat plants grown in the alkaline soil. However, *Pseudomonas* inoculation significantly increased percentage of mycorrhizal infection in tomato and wheat plants grown in calcareous soil. Dual inoculation of tomato and wheat plants with *Pseudomonas* and mycorrhizal fungi improved their growth under calcareous and alkaline soils significantly than other treatments. Results evidenced that the symbiotically effectiveness of AM fungi was affiliated to the associated bacterial species. The GPRBs evaluated did not decrease plant growth or nutrients content in any mycorrhizal plant.

In summary, a synergistic interaction between Glomus spp. and certain strains of GPRB on the plant growth has been no longer lost and thus, the use of combined inoculations in horticultural and field crops to maximize the contributions of plant growth is trenchant.

Keywords : Arbuscular mycorrhizal (AM) fungi, Gowth promoting rhizobacteria (GPRB), Symbiotically effectiveness, Tomato plants, and wheat plants.

The various microorganisms found ordinarily in the rhizosphere and known to improve soil fertility and crop yields embody arbuscular mycorrhizal fungi (AMF), free nitrogen-fixing bacteria and plant growth promoting rhizobacteria (PGRB), such as rhizobia and pseudomonades (O'Gara *et al.*, 1994). The arbuscular mycorrhizal fungi (AMF) are one of the most important components of the soil microbial community. They interact symbiotically with the roots of about 80% of all plant species (Bonfante & Peritto, 1995). Mycorrhizal symbioses are present in most natural and agricultural ecosystems, where they are elaborated in many key processes including nutrient uptake and nutrients cycling, soil conservation of soil fertility, plant soundness and enhancement of nitrogen fixation by rhizobia (Ramadan & Attia, 2002 and Varma & Hock, 1995). In a soil ecosystem, external AM hyphea interact with other soil microorganisms, either directly or indirectly by modifying host physiology root exudation (Azcon-Aguilar & Barea 1992). On the other hand, the behaved of mycorrhizal fungi could be influenced by other root symbionts or by PGRBs (Linderman, 1992). A variety of interactions between arbuscular mycorrhizal (AM) fungi and soil microorganisms have been studied, including interactions with plant pathogens, nitrogen-fixing microorganisms, phosphate-solubilizing microorganisms. On the other hand, influence of the rhizosphere microflora on mycorrhizas is still poorly understood, although positive impacts of the microflora on mycorrhizal root colonization and plant growth have been reported. The contribution of AM fungi to plant growth in a non-sterile soil might differ from that demonstrated in a sterilized one. However, it is extremely difficult to assess the contribution of AM fungi to plant growth in non-sterile soil.

The interactions between functional groups of soil microflora are a key to understanding the dynamic processes that depict plant-soil relationships. Among those, the effects of rhizobacteria on the development and functioning of arbuscular mycorrhizal (AM) fungi (Linderman & Paulitz, 1990) are of notable interest because the latter form a living link between roots and soil (Bethlenfalvay & Schuepp, 1994). AM fungi, in turn, affects the composition of bacterial communities (Paulitz & Linderman, 1991 and Attia & Badr El-Din, 2000), and fungi and bacteria in the mycorrhizosphere are thought to evoke in concert such plant responses as resistance to stress and diseases (Bethlenfalvay, 1992; Linderman, 1992 and Badr El-Din *et al.*, 2000).

The effects of mycorrhizal plant and some strains of PGRB on symbiotic effectiveness were investigated apart. Even though an apparent concept of mycorrhizal (symbiotic) effectiveness is still lacking. In the present study,

mycorrhizal effectiveness was used as an adjustment of the term mycorrhizal dependency as defined by Plenchette *et al.* (1983), based on the relationship between dry mass of plants inoculated with mycorrhizal fungi and the dry mass of un-inoculated plants. Here, the term is used for three parameters (biomass, N-uptake and P-uptake) to describe mycorrhizal (symbiotic) effectiveness. When mycorrhizal effectiveness >0, the mycorrhizal association is considered beneficial for the plant (Heijden and Kuyper , 2001).

The aim of the present work , was to evaluate interactions between mycorrhizal fungi and some growth promoting rhizobactera (GPRBs) namely, *Pseudomonas* sp., *Azotobacter* sp., *Klebsiella* sp. *Azospirillum* sp., *Bacillus* sp., *Enterobacter* sp. on plant grown in a calcareous and an alkaline soil. The effect of GPRBs on mycorrhizal symbiosis was assessed using a tomato and wheat.

Material and Methods

Microorganisms

The microorganisms used were *Pseudomonas putida, Azotobacter chroococcum, Klebsiella* sp., *Azospirillum lipoferum, Bacillus megatherium* and *Enterobacter* sp. These strains were obtained from culture collection of Agricultural Microbiology Dept., NRC, Giza, Egypt.

Mycorrhizal inoculum

Mycorrhizal spores used in this study were mixtures of *Glomus* spp. (*G. mosseae* and *G. fasciculatus.* These spores were originally extracted by a wet sieving and decanting technique (Gerdemann & Nicolson, 1963) from rhizosphere soil of maize and alfalfa (grown in calcareous or alkaline soil) and multi-plated in pot cultures containing a peat: vermiculite: perlite mix 1:1:1 by volume with maize and onion grown for 4 months (Badr El-Din *et al.,* 1999).

Soils and plants

Soil samples were collected from El-Amria and Kafr El-Sheikh. All soil samples were air dried, passed through a four mm-mesh sieve. Some physical-chemical properties of soils are shown in Table 1. Tomato (*Lycopersicon esculentum* va. supermarmand) and wheat (*Triticum aestivum* va. sids 10) were used as the test plants.

TABLE 1. Mechanical physico-chemical properies of the calcareous and alkaline soils (Oven dry basis).

Properties		Calcareous soil	Alkaline soil
Mechanical analysis			
Sand	%	53.4	26.5
Silt	%	29.0	45.0
Clay	%	17.60	38.5
Texture		Loam	Silt clay loam
Chemical analysis			
pH		8.20	8.80
O.C.	%	1.29	1.5
EC	d cm^{-1}	1.41	3.01
$CaCO_3$	%	42.73	1.30
Total N	ppm	181	50
Total P	ppm	190	210
Soluble P	ppm	8.0	4.5

Tomato and wheat seeds were grown in plastic trays, 160 eyes in each, filled with peat moss, enriched with 5 gm activated charcoal and 15 gm calcium carbonate per 100 gm (the pH value ranged between 6.8-7.0). Each eye received 2 ml from the respective microbial culture and 1 g of mycorrhizal inoculum. Healthy seedlings were transplanted to the experimental pots 25 days after sowing.

Twenty kilograms of calcareous or alkaline soil was packed in each of a sufficient numbers of plastic pots (35 x 30 cm). Tomato and wheat seedlings were transplanted to plastic pots with the surrounding peat moss. The pots received half doses of recommended mineral fertilizers, whereas control pots (non-mycorrhizal plant) received full doses of recommended mineral fertilizers. Each treatment was replicated three times.

Sampling and chemical determinations

Plants grown under the various treatments were sampled at flowering stage. Adhering soil was washed gently from the root mass. Shoots and roots were dried at 75°C for 48 hr and weighed. A portion of the root mass (0.5g), sub-sampled for colonization by AM fungi. The root was cleared in 10% KOH overnight and stained with trypan blue (Attia, 1999). The percentage of root infection with AM fungi was evaluated using the magnified intersect method described by McGonigle *et al.* (1990).

Calculation of symbiotic effectiveness

Symbiotic (mycorrhizal) effectiveness was calculated for (1) shoot biomass,

(2) N-uptake and (3) P-uptake as: 1-(b/a), where a is the mean plant biomass or N- or P-uptake of one of the mycorrhizal inoculated treatments, and b is the mean plant biomass or N- or P-uptake of uninoculated control (Heijden & Kuyper, 2001).

Statistical analyses

One-way analyses of variance and T-tests were done on the data using the SPSS (Statistical Package for the Social Sciences) system. All means were tested for significance using the Duncan's multiple range tests at the 5% level for probability.

Results and discussion

Mycorrhizal colonization

Inoculation with mycorrhizal fungi increases AMF colonization of roots significantly (Table 2). This indicates that propageus of the native AM fungi was insufficient. Mycorrhizal colonization was significantly enhanced by bacterial inoculation (Table 2). Mycorrhizal colonization of tomato and wheat plant was overwhelmed by soil type and bacterial inoculation. *Azotobacter* inoculation exercised lowest effect on mycorrhizal colonization of tomato in soil I but not in soil II, while inoculation with *Pseudomonas* enhanced mycorrhizal colonization in both wheat and tomato plants in both soil types. GPRB inoculation stimulated mycorrhizal colonization of tomato plants grown in soil I compared to those grown in soil II, but had a similar effect on roots of wheat plants grown in soil I and II.

TABLE 2. Mycorrhizal colonization of tomato and wheat plants grown in calcareous or alkaline soils and inoculated with GPRB's

Treatments	Tomato		Wheat	
	Soil I	Soil II*	Soil I	Soil II
NPK	20 a	17 a	15 a	14 a
AMF	72 bc	61 bc	61 bc	63 b
AMF+Az	73 bc	65 bc	67 c	62 b
AMF+Azot	62 b	52 b	60 bc	54 b
AMF+ Ent	71 bc	60 bc	54 b	52 b
AMF+Kleb	75 cd	67 bc	57 b	67 b
AMF+Ps	84 d	71 c	73 c	73 c
AMF+B.	76 cd	70 c	61 bc	63 b

Values within a column that are followed by a different letter indicate significant differences between treatments at P<0.05 according to Duncan's multiple range tests and values strict by * indicate significant differences between soil types.

A similar strain effect has been observed previously for early stages of root colonization by mycorrhizal fungi (Meyer & Linderman, 1986b; Gryndler & Vosatka, 1996 and Attia, 1999) . There are several possible mechanisms for the stimulatory effects (Gryndler & Vosatka, 1996). Bacteria might exudate some biologically active molecules that directly or indirectly (*via* plant physiology) affect the mycorrhizal fungi. Fluorescent pseudomonades (like *Ps. Putida*) produce numerous metabolites, including plant growth regulators such as auxins, gibberellins, and ethylene, biotin, nicotinic acid and pantothenic acid, which affect the growth of plants and microorganisms in soil (Hussain & Vancura, 1970 and Awad 1998). The production of physiologically active concentrations of indole-3-acetic acid and some other auxin molecules has been reported in *Pseudomonas* and *Azospirillum* (Prikryl *et al.*, 1985 and Awad, 1998). On the other hand, bacteria might affect root cell walls, thereby increasing susceptibility of plant tissue to fungal penetration (Will & Sylvia, 1990). *Azospirillum brasiliense* produces pectolytic enzymes in vitro which soften root cell walls in the soil (Umali-Garcia *et al.*, 1980). *Klebsiella* sp. might produce a volatile substance which stimulates hyphal extension (Will and Sylvia, 1990).

Plant growth

Inoculation with AM fungi significantly increased shoot and root dry weight of tomato plants grown in soil II and roots dry weight of wheat plants grown in soil I. On the other hand, chemical fertilizers significantly increased shoot dry weight of tomato plants grown in soil I and root and shoot dry weight of wheat plants grown in soil II and I, respectively (Table 3).

Simulative effects of mycorrhizal PGPR inoculation on tomato were more prominent in soil I than in soil II (Table 3), whereas the dual effect of mycorrhizal fungi and GPRB on wheat plants was observed in both soils. In general, growth of mycorrhizal tomato plants was worse under all GPRB strains except in cases of inoculation with *Azotobacter* or *Azospirillum* compared with those inoculated with *Pseudomonas* in soil II. Mycorrhizal wheat inoculated with *Pseudomonas* or *Azotobacter* produced more root dry weight relative to other treatments on soil I than soil II.

N and P in shoots

N and P uptake in tomato plants were stimulated mainly by bacterial and AM fungi inoculation. In soil I mycorrhizal tomato plant inoculation with all GPRB

strains significantly increased N uptake except for *Azospirillum* and *Pseudomonas* in soil I. AM fungi inoculation in combinations with GPRB led to a significant increase in plant P uptake in soil I, while in soil II dual action of AM fungi plus *Azotobacter* was superior to other treatments. The increase in P uptake was the same as mineral fertilizers. On the other hand, no significant differences were detected in N uptake of mycorrhizal wheat plants plus all GPRB strains, except for *Pseudomonas* in soil I. However, for soil II, significant increases due to AM fungi associated with *Klebsiella* and *Bacillus* were apparent. The rest of the strains behaved the same (Table 4).

In soil I, highest P uptake was noted in plants inoculated with AM fungi plus *Klebsiella* compared to mineral fertilizers. However, in soil II, significant increases in P uptake of mycorrhizal wheat plants with all GPRB strains compared to mineral fertilizers were distinct (Table 4).

Ravnskov & Jakobsen (1999) found that dual inoculation with *G. intraradices* and *P. fluorescens* DF57 did not lead to a synergistic effect on P uptake by plants. However, a synergistic effect of dual inoculation with a multi-strain mix of different species of AM fungi and *P. putida* was observed on P concentrations in plants by Meyer & Linderman (1986b). These dissimilar results might be related to the use of different *Pseudomonas* species. Will & Sylvia (1990) found that there was no consistent evidence for a synergistic effect of dual inoculation with *Klebsiella* sp. and AM fungi on sea oat growth. Bagyaraj & Menge (1978) reported that there is a synergistic or additive beneficial effects on tomato plants grown in sterilized as well as in unsterilized soils when plants were inoculated with both *Glomus* spp. and *Azotobacter* sp.

Symbiotic effectiveness

Mycorrhizal effectiveness (biomass) was relatively constant positive in soil II and usually slightly negatives in soil I (for tomato inoculated with *Azospirillum* and mycorrhizal wheat inoculated with all bacterial inoculation). Mycorrhizal effectiveness of mycorrhizal tomato plants was highest for *Azotobacter* in soils I and with *Azotobacter* and *Azospirillum* in soil II and lowest for *Pseudomonas* and Bacillus. In mycorrhizal wheat, mycorrhizal effectiveness was highest for *Klebsiella, Bacillus*, and lowest for *Pseudomonas* (Fig 1).

TABLE 3. Shoot and root dry weights of mycorrhizal tomato and wheat plants grown in calcareous and alkaline soil and inoculated with GPRB.

Treatments	Tomato				Wheat			
	Root		Shoot		Root		Shoot	
	Soil I	Soil II*	Soil I	Soil II*	Soil I	Soil II	Soil I	Soil II
NPK	0.62 a	0.43 a	3.07 b	2.45 a	0.27 a	0.26 d	2.78d	1.47 a
AMF	0.69 ab	0.55 bc	2.33 a	2.71 b	0.44 d	0.21 bc	2.49bc	1.50 a
AMF+Az	0.72 b	0.59 cd	2.58 ab	3.00 c	0.38 c	0.25 cd	2.55c	1.69 c
AMF+Azot	0.97 b	0.50 ab	4.47 c	1.01 c	0.37 c	0.34 e	2.25a	1.67 b
AMF+ Ent	0.92 b	0.65 d	4.13 c	2.70 b	0.34 bc	0.20 ab	2.53c	1.71 c
AMF+Kleb	0.89 b	0.56 bc	4.20 c	2.66 ab	0.45 d	0.17 a	2.54c	1.08 d
AMF+Ps	0.97 b	0.57 bc	3.86 bc	2.84 bc	0.27 a	0.32 e	2.43abc	1.72 c
AMF+B	0.98 b	0.62 cd	3.84 c	2.67 ab	0.30 ab	0.23 cd	2.31ab	1.10 d

Values within a column that are followed by a different letter indicate significant differences between treatments at P<0.05 according to Duncan's multiple range tests and values strict by * indicate significant differences between soil types.

TABLE 4. Shoot N and P uptake (mg plant^{-1}) of mycorrhizal tomato and wheat plants inoculated with GPRB grown in calcareous and alkaline soils.

Treatments	Tomato				Wheat			
	N		P		N		P	
	Soil I	Soil II	Soil I	Soil II	Soil I	Soil II	Soil I	Soil II
NPK	131.62abc	18.13a	2.37b	0.67a	103.4a	15.6a	2.89ab	0.93a
AMF	89.83a	26.82ab	1.54a	0.85a	101.2a	21.5b	2.49b	1.24b
AMF+Az	109.80ab	35.87b	2.48b	1.72b	99.7a	21.9b	4.19c	1.85d
AMF+Azot	223.04e	32.26b	4.18c	2.18c	92.6a	22.0b	3.21ab	1.39bc
AMF+ Ent	205.55de	33.27b	4.23c	1.27b	125.2a	22.2b	2.94a	1.86d
AMF+Kleb	177.35cde	35.65b	4.74c	0.76a	105.5a	34.1d	4.20c	2.07de
AMF+Ps	141.86abc	35.86b	4.03c	0.84a	133.8b	21.5b	3.06ab	1.52c
AMF+B	161.35bcd	28.45ab	4.32c	0.84a	95.3a	30.9c	2.39ab	2.13e

Values within a column that are followed by a different letter indicate significant differences between treatments at P<0.05 according to Duncan's multiple range tests and values strict by * indicate significant differences between soil types.

Mycorrhizal effectiveness (N-uptake) indicated a similar pattern as mycorrhizal effectiveness (plant biomass) in both soil, except for mycorrhizal wheat inoculated with *Azospirillum*, *Azotobacter* and *Bacillus* in soil I. However, mycorrhizal effectiveness (P-uptake) was highest in both soils trailed. For all mycorrhizal tomato, mycorrhizal effectiveness was higher in case of inoculation with all tried bacteria than for mycorrhizal wheat (Fig. 1).

Results evidenced that the symbiotically effectiveness of AM fungi was affiliated to the associated bacterial species. The GPRBs assayed did not decrease plant growth or nutrients content in any mycorrhizal plant. These results are confirming with those looked to previously by Barea *et al.* (1998),

who found that certain *Pseudomonas* strains that produce DAPG (2,4-diacetyl-phloroglucinol) and that are used as biological agents do not exhibit detriment effects on AM fungus *G. mosseae*. This, however, might be based on the assumptions that no bacterial treatment adversely affected mycorrhizal colonization and even with the DAPG over producer F113 (PCU203) the mycelia development was not significantly less than the mycelia development when no bacteria were introduced (Barea *et al.*, 1998). A positive effect of bacterial-fungi inoculate was evidenced in some cases.

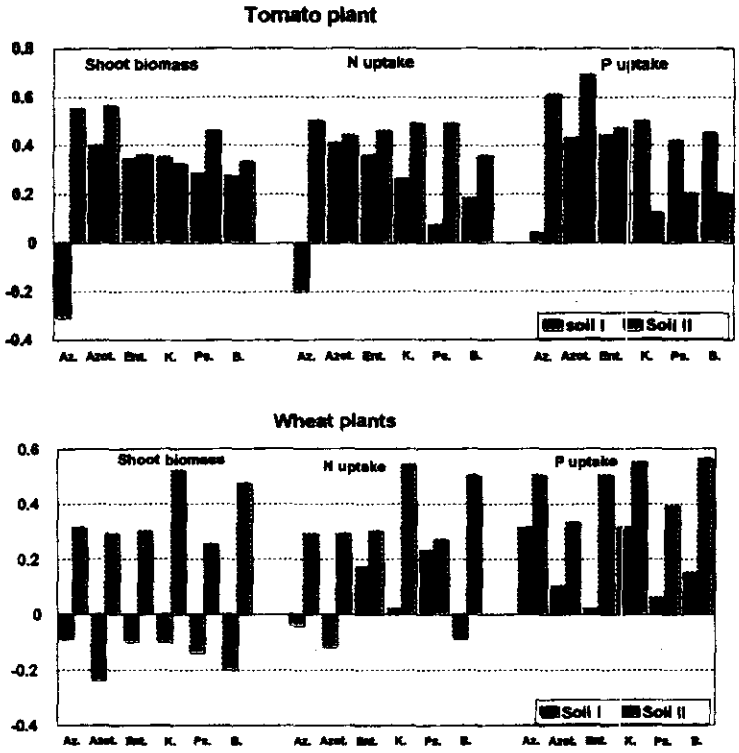

Fig. 1. Mycorrhizal effectiveness based on shoot dry weight, shoot N-uptake and P-shoot uptake of mycorrhizal tomato and wheat plants inoculated with GPRB grown in calcareous and alkaline soils .

The most effective bacterial inoculum on plant growth was; *Pseudomonas* in mycorrhizal tomato plant grown in soil I and *Azospirillium* in mycorrhizal wheat plant grown in soil II (Table 3). But no significant differences were found in N

and P uptakes in mycorrhizal tomato plant inoculated with *Pseudomonas* and N uptake in mycorrhizal wheat plant inoculated with *Azospirillium* (Table 4). It seems reasonable to state that phosphorus uptake might be the relevant factor. It is conceivable that, as the verification of the infection is preceded by the same fungus growth, such as propageus germination or pre-infective hyphal elongation, AM could be stimulated by bacteria before it comes into contact with root cells (Azcon, 1987). Symbiotical effectiveness can also be stimulation on elongation, distribution, or surviving of external post-infective mycelium. Any of these nominees can increase AM symbiotical effectiveness. These effects could be motivated by a direct bacterial action on AM fungi or through the host plant. The bacteria might affect plant growth by the plant auxins which they synthesis. These growth substances of the auxin, gibberellin and cytokinin types also could be caught up in the microbial interactions. The study of these microbial groups must be considered in conjunction. This is necessary for a better knowledge and understanding of such interactions in order to utilize AM fungi successfully.

In summary, a synergistic interaction between *Glomus* spp. and certain strains of GPRB on the plant growth has been no longer lost and thus, the use of combined inoculations in horticultural and field crops to maximize the contributions of plant growth is trenchant. The synergistic effect on plant growth has been accepted. The study of such combinations under field conditions will be the aim of future research.

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(*Received* 8/7/2002;
accepted 23/3/2003)

تقييم التاثير التّعايش لفطريات الميكوريزا ببعض البكتريا المحفزة لنمو النبات

مجدى عطية ونعمت مصطفى عوض
قسم الميكروبيولوجيا الزراعية - المركز القومى للبحوث - الدقى- القاهرة -مصر .

أجريت تجربة اصص لتقييم تأثير خمسة سلالات من الميكروبات المحفزة لنمو النباتات (أزوسبيريلوم ، إنتروباكتر، كليبسيلا، بسيودوموناس أو باسيلس) على نشاط وكفاءة فطر الميكوريزا وتشكيلاته وأيضا على بعض معايير نمو نباتات القمح والطماطم النامية في تربة جيرية أو قلوية . اشارت النتائج بأن السلالات المختبرة زاد فيها الوزن الجاف لجذور والمجموع الخضرى لنباتات الطماطم الملقحة بالميكوربزا النامية فى كلا من التربة الجيرية و القلوية بالمقارنة بالتلقيح المنفرد لفطريات الميكوربزا. وقد اظهرت النتائج زيادة فى الوزن الجاف لنباتات القمح فى التربة الجيرية بالمقارنة بالتربة الغير ملقحة ، بينما لم يظهر التحليل الاحصائى فروق معنوية فى الوزن الجاف لنباتات القمح النامية فى ألتربة القلوية الا انه أظهر زيادة معنوية فى النيتروجين والفوسفور الممتص بالمقارنة بالنباتات الغير ملقحة في كلتا الترب.

لم يظهر تأثير للتعايش ا بين فطر الميكوريزا والسلالات الميكروبية المختبرة على النسبة المئوية لإصابة جذور الطماطم بفطريات الميكوريزا ولا الأعداد الكلية لجراثيم الفطر فى المنطقة المحيطة بجذور الطماطم والقمح النامى في التربة القلوية. بينما أظهر التلقيح بالباسيدوموناس والميكوربزا زيادة معنوية فى النسبة المئوية لإصابة جذور الطماطم والقمح النامى فى التربة الجيرية. وكذلك تحسن نمو كلا من النباتاتين النامى فى التربة الجيرية والقلوية بالمقارنة بالمعاملات الاخرى .

وقد اكدت النتائج زيادة نشاط الميكوريزا وكفائتها عند تلقيحها مع البكتريا المحفزة لنمو النبات ولم يظهر نقص فى نمو النباتات أو المحتوى الغذائى فى النباتات الملقحة بالسلالات المختبرة على النباتات المصابة بالميكوريزا .