INTERACTION BETWEEN VIRUS INFECTION AND FUNGAL DISEASES IN BEAN PLANTS (*PHASEOLUS VULGARIS*) : II- EFFICIENCY OF MYCORRHIZAL INOCULATION ON EFFECTIVNESS OF BEAN COMMON MOSAIC VIRUS (BCMV) AND *RHIZOCTONIA SOLANI*.

M. H. ABD EL-MAGEED * AND R. A. ZAGHLOUL **
* FUNGUS AND PL. PATHOL. BRANCH,
** MICROBIO. BRANCH,
AGRIC. BOT. DEPT., FAC. AGRIC., MOSHTOHOR, ZAGAZIG UNIV., EGYPT.

The efficiency of mycorrhizal inoculation (*Glomus macrocarpum*) on effectiveness of BCMV and *R. solani* infection was studied in bean plants (*Phaseolus vulgaris* L.). Results showed that *R. solani* when introduced alone in sterilized soil caused the highest percentage of post-emergence damping-off and root-rot diseases and the treatments using mycorrhizal or BCMV inoculation decreased the percentage of fungal diseases while, the lowest percentage of fungal infection resulted from the combination of VAM fungus and viral inoculation. The highest percentage of viral infection occurred with VAM fungus inoculation while, *R. solani* decreased the percentage of viral infection. Moreover, the lowest percentage of viral infected plants occurred with the combination of *R. solani* and VAM fungus in sterilized soil. Slight reduction in colonization intensity and spore production of VAM fungus were occurred with viral infection regardless the type of soil. While, the highest reduction was approved with fungal inoculation. Total bacterial counts in the rhizosphere soil were higher in case of inoculation with VAM and BCMV combination at all growth periods VAM fungus when inoculated alone are combined with BCMV increased the counts of Actinomycetes at all growth periods of bean plants. On the contrary, viral and mycorrhizal inoculation decreased the counts of fungi in soil specially in the combination of VAM fungus and BCMV treatment which led to sharp decrease in population of fungi.

VAM fungus treatments gave highly increase in plant height, number of leaves/plant, fresh and dry weights of roots and shoots and No. of flowers/plant when inoculated alone as well as when associated either with *R. solani* regardless the type of soil. However, all growth characters were decreased in case of viral or fungal infection in sterilized and non-sterilized soils.

Also, obtained results indicated that total carbohydrates, protein content, nitrogen, phosphorus and potassium were increased with VAM fungus treatments. Chlorophyll A was almostly proportionated with carbohydrates except in case of BCMV. The VAM fungus gave the highest level of chlorophyll B while, chlorophyll C increased with all treatments except in plants inoculated with BCMV and VAM fungus in non-sterilized soil.

Bean (*Phaseolus vulgaris* L.) is considered to be one of the most important feeding crops in Egypt and area of bean crop had increased to 18225 feddan of dry bean in the season 1994 **(Anon, 1995)**

Under field conditions, beans suffer from several diseases such as rusts root-rot and damping-off as well as viral diseases, causing considerable losses in the yield and quality. In addition, bean plants may be infected with more than one disease **(Dixon, 1981)**

R. solani is considered to be one of the most important pathogens which caused damping-off and root-rot diseases on bean, cowpea, broad bean and lupine crops **[Brown, 1959; Abd El-Mageed, 1981; Omer, 1986 and Gowily, (Ahlam) 1987]**

In Egypt, several viruses specially Bean Common Mosaic Virus (BCMV) have bean reported to infect beans **(Deif, 1977; Omar *et al.*, 1982 and Abd El-Mageed, 1986)**. On the other hand, virus infection was reported either to increase fungal infection **(Reyes and Chadna, 1972; Beniwal and Gudawskas, 1974; and Salt, 1982)** or to decrease some other fungal infections **(Magyarosy and Hancock, 1974; El-Hammady *et al.*, 1983; Gamal El-Din *et al.*, 1990; and Abd El-Mageed,1986 and 1992)**

Mycorrhizal fungi was reported to decrease or inhibit soil-borne fungal diseases by **Zamblin and Schenck (1983), Garcia-Garrido and Ocampo (1987), Schnobeck (1987), Kope and Fortin (1990) and Eisa (Nawal) *et al.* (1994)** However, some reports indicate an increase in disease severity under the influence of VA-Mycorrhizal fungi **(Davis and Menge, 1980)**

As regards to the effect of VAM on viral infection, **Schnobeck and Schinzer (1972)** reported that, inoculated tobacco plants with TMV obtained more lesions on leaves of mycorrhizal inoculated than non-mycorrhizal inoculated plants. **Daft and Okussanya (1973)** demonstrated that, the increase in virus titer associated with mycorrhizal (*Glomus macrocarpum* var *geosporus*) inoculated plants due to increase plant organs phosphate level

The aim of this study, is to investigate the efficiency of mycorrhizal inoculation on the activity of BCMV and *R. solani* infection in bean plants in sterilized and non-sterilized soil and their effects on some growth characters and chemical constituents of bean plants

MATERIALS AND METHODS

Source of pathogenic agents :

1- The fungus :

Two isolates of *Rhizoctonia* (*Corticum*) *solani* Kuhn were isolated from damping-off and root-rot of naturally infected bean plants (*Phaseolus vulgaris* L.) collected from the farm of Fac. Agric. Moshtohor, Zagazig Univ. Purification of the

two isolates was carried out using hyphal tip technique then identified at Plant Pathology Institute, Agricultural Research Center, Giza, Egypt.

2- The virus :

Bean common mosaic virus (BCMV) was obtained from naturally infected bean plants and identified according to host range, differential hosts, transmission and physical properties and continually maintained in a freezing infected leaves collected from recently inoculated plants.

Source of Vesicular Arbuscular Mycorrhiza (VAM):

Glomus macrocarpum, soil Goettinge strain was obtained from Tropical Institute, Goettingen University, Fedral R. of Germany by **El-Deepah (1981).**

Determination of the Aggressive Isolated Fungi and Inoculum Potential:

The inoculum of the two isolated fungi was grown on sterilized sand sorghum grain medium **(Whithead, 1975)**. Clay pots (ϕ 30 cm) were sterilized properly using 5% formaline solution. Clay loam soil was autoclaved at 15 lb./inch^2 for 3 hours then infested with different amounts of inoculum *i.e.* 0.5, 1.0, 3.0, 5.0 and 8.0% of soil weight. Ten bean seeds of Giza-3 cv. were sown in each pot with three replicates.

Post-emergence damping-off and root rot were recorded 15-45 days after sowing. The aggressive isolate and potential inoculum rate were chosen according to obtained results and were subjected in further study.

Fungal soil infestation and Mycorrhizal Inoculum Propagation :

Sterilized soil was infested with inoculum of *R. solani* at a rate of 3% of soil weight. Sterilized water was applied to the soil and all were thoroughly mixed to ensure even distribution of fungal inoculum, then left for one week for fungal activation. Sterilized non-inoculated sorghum grain medium was added to control pots.

The VAM fungus (*Glomus macrocarpum*) was propagated on onion plants using the propagation technique described by **Al-Fassi *et al.* (1990)**. After three months from onion cultivation, the mycorrhizal roots of onion bulbs together with its adjacent soil were collected and used for mycorrhizal infestation.

The mycorrhizal inoculum contained of infected onion roots and its rhizospheric soil added just before sowing at a rate of 10 g/pot (each pot contains 5kg of soil).

Cultivation process :

Ten surface-sterilized bean seeds Giza-3 cv. were sown at a depth of nearly 2 cm in each pot. Six pots were used as replicates for each treatment in a randomized complete block design and kept under insect proof greenhouse. Three replicates were remained to the end of experiment for growth characters and chemical analysis while, the others were used in periodical analysis i.e. microbiological and pathological determinations. All pots have been supplied with the equal amounts of N and P as

ammonium sulphate and super phosphate at a rate of 30 kg N and P_2O_5 per feddan in two equal doses at vegetative and flowering stages.

Seedlings of two-weeks-old that grown in uninfested or infested as well as natural soils were thinned to 5 apparently healthy ones per pot. Viral inoculation was carried out by rubbing carborandum dusted leaves as quickly as possible with BCMV infectious sap. Inoculated leaves were rinsed with tap water. All plants were kept under insect proof greenhouse.

Treatments of the designed combined fungal, mycorrhizal and viral infections in infested and natural soil were as follows

A- Sterilized Soil :

- Control (1) no mycorrhiza, no *R. solani* and no viral infection.
- Fungal infection (*R. solani*).
- Viral infection (BCMV).
- Mycorrhizal inoculation (*Glomus macrocarpum*)
- Fungal and viral infection.
- Fungal and mycorrhizal inoculation.
- Mycorrhizal and viral inoculation.
- Fungal,viral and mycorrhizal inoculation.

B- Non-sterilized Soil :

- Control (2) no mycorrhizal and no viral inoculation.
- Viral infection.
- Mycorrhizal inoculation.
- Mycorrhizal inoculation and viral infection.

Determinations :

A- Disease assessment.

1. Percentage of post-emergence damping-off. The percentage of dead emerged seedlings was determined.
2. Root-rot of diseased plants were determined by using plants 45-days-old carefully removed, washed currently with tap water and examined for root-rot symptoms. Determination of the root-rot disease severity index (DSI) was carried out based on a scale from 0 (non visible damage) to 5 (completely destroyed roots) according to **Salt (1981)**.
3. Percentage of viral infected plants.
4. Plants artificially inoculated with BCMV, were continuously observed for virus symptoms and on basis of based number systemically infected bean plants out of inoculated ones. The percentage of virus infected plants was calculated.
5. For each treatment into which the VAM fungus was included, the intensity of root colonization with such fungus was determined using the method described by **Phillips and Hyman (1970)**.

B- Microbiological Determinations :

Total bacteria, actinomycetes and fungi were counted in the rhizosphere region for non-sterilized soil treatments biweekly during the course of experiment. The soil extract yeast agar medium was used for counting the total bacteria **(Skinner *et al.*, 1952)**. Jensen's medium was used for actinomycetes count and prepared as described by **Allen (1950)** while, **Martin's medium (1950)** was used for counting the total fungi. The plates count method was used for microbial determinations.

C- Plant Growth Parameters :

1. Plant height (cm).
2. Leaves number/plant.
3. Flowers number/plant.
4. Fresh and dry weights of root system/plant.
5. Fresh and dry weights of shoot system/plant.

D- Chemical analysis :

1. Chlorophyll A and B as well as carotenoides were estimated in the 3rd leaf of the plant according to **Wettstein (1957)**.
2. Total carbohydrates content was determined in dry matter of leaves by the phenol sulphuric acid method described by **Michel *et al.* (1956)** and calculated as mg/g dry weight.
3. Total nitrogen was determined in the dry matter of shoot system by using wet digestion according to **Piper (1947)** and using micro-Kjldahl as described by **Pregl (1945)**. Then the crude protein was calculated according to the following equation :
4. Crude protein = Total nitrogen X 6.25 **(A.O.A.C., 1975)**.
5. Total phosphorus was determined in the dry matter of shoot system colourimetrically according to **American Public Health Association (1989)**.
6. Total potassium was estimated in the dry matter of shoot system by flame photometer apparatus according to the method described by **Brown and Lilliland (1946)**.

RESULTS AND DISCUSSION

Pathogenecity and Inoculum Potential of *R. solani* Isolates on Bean Plants.

Data in Fig. (1) show that, isolate (I) of *R. solani* was more aggressive than the isolate (II). The percentage of post-emergence damping-off and root-rot were increased with increasing the inoculum potential of the two isolates. This agreed with many earlier investigators **(Khan, 1966; Abd El-Kadir, 1977 and Omer, 1986)** who indicated that on the basis of differences in pathogenesis, *R. solani* was the most virulent in causing post-emergence phase and root-rot disease and these diseases increased with increasing the inoculum potential. So, the isolate (I) was chosen to carry out this research.

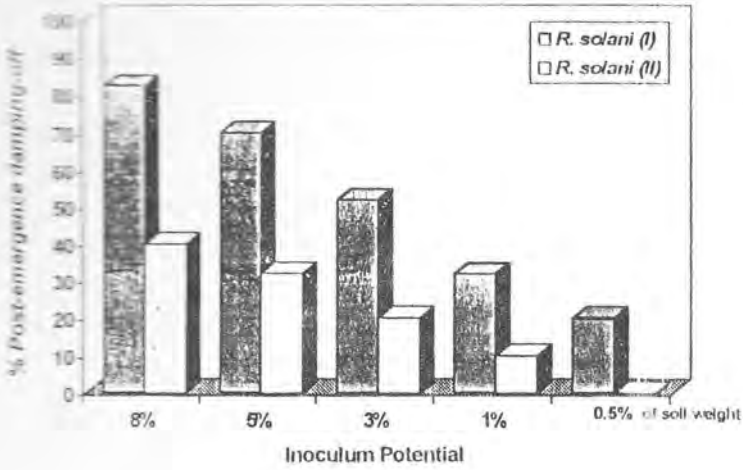
Effect of Viral, Fungal and Mycorrhizal Inoculation on Disease Severity and VAM colonization :

Data in Table (1) show that, *R. solani* in sterilized and non-sterilized soil treatment caused the highest percentage of post emergence damping-off and disease seventy index (DSI) of root-rot compared with other treatments. These results are in agreement with several investigators (**Omer, 1986 and Gowily , 1987**). Also, data show that, viral infection or VA-mycorrhizal inoculation decreased the percentage of damping-off and (DSI) of root-rot. The lowest percentage of fungal infection resulted from using the combination of VAM fungus and viral inoculation plus Rhizoctonia inoculation and this result was also true in sterilized soil. These results could be attributed to an antagonistic effect between fungal and viral infection and/or to Mycorrhizal effect, **Abd El-Mageed (1986 and 1992)** found that, soluble and cell wall bounds protein extracted from hypocotyle, leaves, pods and roots of viral infected plants contained more polygalcturinase inhibitor than proteins from non-viral infected plants. Moreover, data show that, VA-Mycorrhizal inoculation decreased the percentage of fungal infection which agree with **Zamblin and Schenck (1983), Garcia-Garrido and Ocampo (1987), Kope and Fortin (1990) and Eisa (Nawal) *et al.* (1994)** who reported that, mycorrhizal fungi decrease or inhibit soil-borne fungal diseases.

Symptoms of viral infection in the different included treatments showed the following :

The highest percentage of viral infection occurred in plants sown in sterilized or non-sterilized soil previously inoculated with VAM fungus. Similar results were reported by **Schnobeck and Schinzer (1972)** who found that inoculated tobacco plants with TMV induced more lesions on leaves of mycorrhizal than non-mycorrhizal plants. Percentage of viral infected plants decreased when the virus inoculated alone in non-sterilized soil and clearly decreased when it was combined with *R. solani* in sterilized soil while, the highest decrease in the viral infected plants occurred when *R. solani* was associated with mycorrhizal fungus in sterilized soil which agree with other investigators who found that several fungi decrease viral infection most probably due to antiviral properties of the fungus (**Zink and Duffus, 1975; Allam *et al.*, 1978; El-Hammady *et al.*, 1983 and Abd El-Mageed, 1995**).

As regard to intensity of mycorrhizal colonization in different treatments, it was clearly shown that, the highest percentage of colonization intensity and average number of spores production such as Arbuscular (large spore) Vesicular (small spore) and mycelium occurred with highly number in case of mycorrhizal inoculation alone in sterilized soil. Whereas, all other treatments reduced the colonization intensity regardless the type of soil. Also, the obtained data show that, reduction in colonization intensity and spore production were occurred with fungal (*R. solani*) inoculation. These results are in harmony with **Eisa (Nawal) *et al.* (1994)**. **Jayashree *et al.* (1995)** observed that, Mung Bean Yellow Mosaic Virus (MBYMV) reduced mycorrhizal colonization and spore production in all the tested VAM fungi.



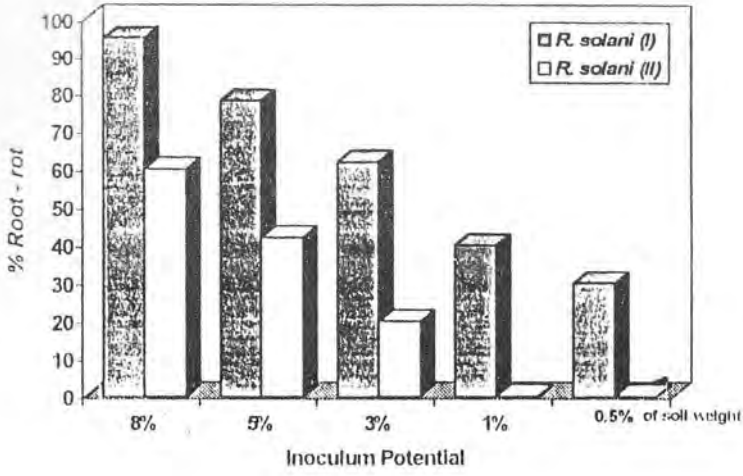


Fig. (1) : Pathogenicity and inoculum potential of *R. solani* isolates on bean plants.

Table (1) : Effect of viral, fungal and VA-Mycorrhizal inoculation on disease severity and VAM colonization in bean.

Parameters / Treatments	Post emergence damping-off (%)	Root-rot (%)	Viral infected plants (%)	Colonization intensity with VAM (%)	Average Number Vesicles	s	Mycelium
A-Sterilized soil							
Control (1)	0.00	-	-	-	-	-	-
R. solani	53.34	60.00	-	-	-		-
Bean common mosaic (BCMV)	0.00	-	80.00	-	-	-	-
G. macrocarpum	0.00	-	-	68.16	13.63	19.2	8.80
R. solani + BCMV	26.67	26.00	23.33	-	-	-	-
R. solani + *G. Macro.*	13.32	38.33	-	30.42	6.70	9.50	4.47
G. macro.+ BCMV	0.00	-	86.67	60.09	10.6	16.44	8.16
R. solani+BCMV+*G. macro.*	6.67	16.00	16.67	43.84	8.37	12.97	6.50
B-Non-sterilized soil							
Control (2)	33.33	36.67	- .	-	-	-	-
BCMV	20.00	13.33	73.33	-	-	-	-
G. macrocarpum	16.67	20.00	-	57.59	12.63	15.6	5.30
G. macrocarpum + BCMV	13.33	12.13	80.00	50.59	10.80	12.36	5.47
L.S.D. at 0.05	15.43	8.75	15.44	6.16	1.37	1.69	0.981
L.S.D. at 0.01	20.97	12.28	21.65	8.64	1.92	2.38	1.375

Table (2) : Effect of viral, fungal and VA-Mycorrhizal inoculation on bean growth characters.

Parameters / Treatments	Plant Height (cm)	No. of leaves /plant	Roots weight Fresh g/plant	Roots weight Dry g/plant	Shoots weight Fresh g/plant	Shoots weight Dry g/plant	No.of flowers /plant
A- Sterilized Soil							
Control (1)	68.33	8.33	1.814	0.479	9.56	1.67	16.00
R. solani	58.33	8.00	0.953	0.474	8.73	1.52	11.33
Bean common mosaic virus (BCMV)	50.00	7.33	1.573	0.354	9.08	1.19	13.33
G. macrocarpum	87.66	11.33	4.06	1.686	11.25	2.68	24.00
R. solani + BCMV	68.33	10.66	1.82	0.417	12.37	1.86	20.66
R. solani + *G. mac.*	98.33	9.66	1.44	0.510	17.51	2.43	14.33
G. mac. + BCMV	78.33	9.66	0.843	0.534	10.59	1.88	19.33
R. solani + BCMV + *G. mac.*	68.33	8.33	0.993	0.540	8.51	0.95	19.00
B- Non-sterilized soil							
Control (2)	61.66	9.33	0.957	0.392	8.92	0.94	16.66
BCMV	46.66	8.00	0.693	0.362	8.63	0.87	12.33
G. macrocarpum	81.66	12.66	1.017	0.466	9.91	1.26	23.33
G. macrocarpum + BCMV	68.33	10.66	0.893	0.342	7.37	0.80	20.66
L.S.D. at 0.05	15.33	1.09	0.508	0.131	1.81	1.08	0.508
L.S.D. at 0.01	20.84	1.46	0.691	0.178	2.82	1.47	0.691

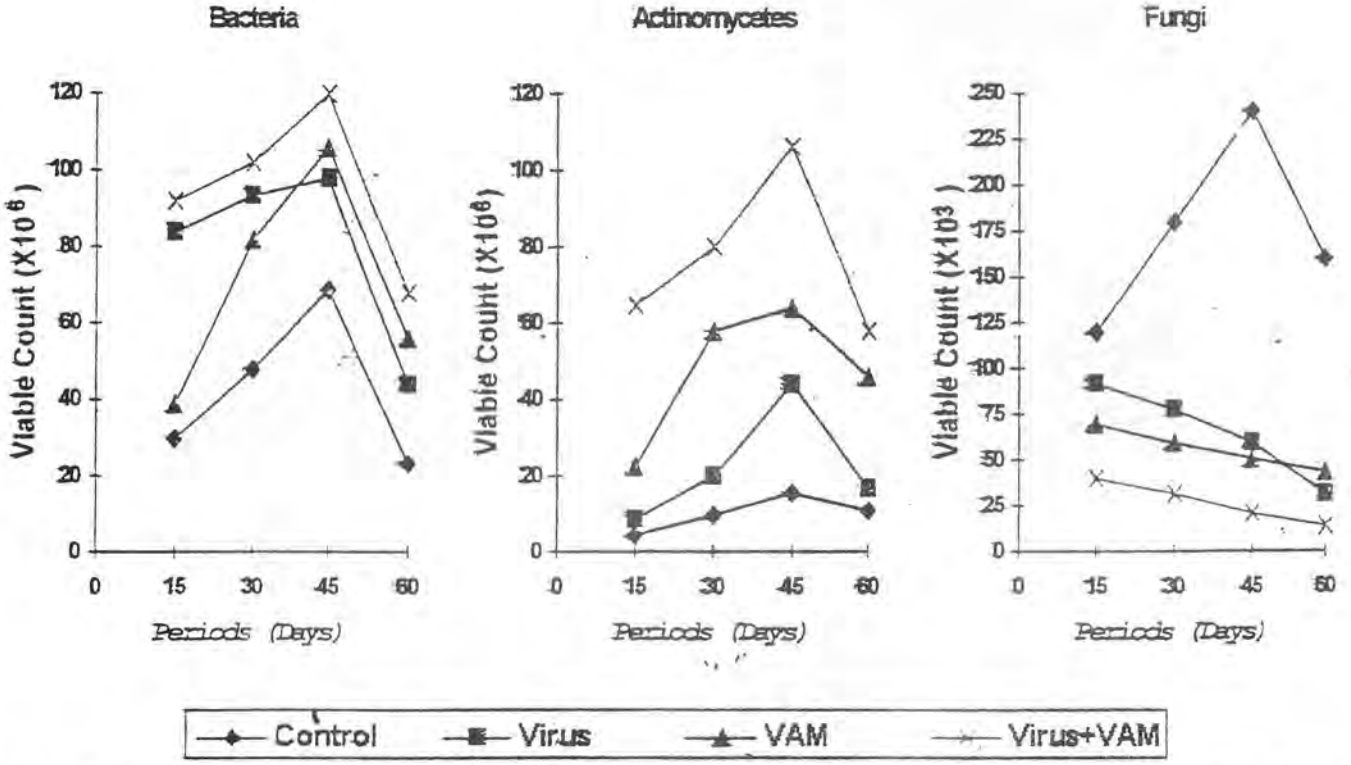


Fig. (2): Periodical changes in bacteria, actinomycetes and fungi counts during growth period of bean plants in non sterilized soil.

Effect of Viral and Mycorrhizal Inoculation on Rhizosphere Microbial Counts :

Data in Fig. (2) show that, the populations of soil bacteria and actinomycetes under various investigated treatments gradually increased with increasing the growth period to reach their maximal values at 45 days and decreased thereafter. On the contrary, except the control treatment fungi counts decreased with increasing the growth period till the end of experiment. Compared with the control, total counts of bacteria in rhizosphere of viral infected plants were higher at the early growth periods of bean plants than VAM fungus treatment, while, the bacterial counts were low at the commencement in case of mycorrhizal plants and increased thereafter till the 45 days of growth period. This may be due to the viral infection leads to increase permeability of cell membranes and leading to release organic substances (carbohydrates, amino acids and protein) which may activate the bacterial proliferation (**Evans and Stephense, 1989**). This result is in harmony with **Abd El-Mageed (1992)** who found that the total bacterial count in rhizosphere of virus infected plants at flowering stage of bean was higher than the rhizosphere of healthy plants. Inoculation of bean plants with BCMV and *G. macrocarpum* enhanced the bacterial proliferation and the bacterial counts were higher at all growth periods than other treatments and this effect may be due to the improvement in nutritional requirements for bacterial growth in case of mycorrhizal inoculated plants. In addition, data in Fig. (2) emphasize that, actinomycetes counts increased in case of viral infection and mycorrhizal inoculation compared with the control and the increase of actinomycetes counts in these treatments may be reflected on the reduction of post emergence damping-off and root-rot diseases in case of BCMV and VAM fungus treatments which previously discussed in Table (1). VAM fungus *G. macrocarpum* when inoculated alone or combined with BCMV increased the counts of actinomycetes and this was recorded at all growth periods of bean plants. This result could be attributed to the mycorrhizal fungi produced growth promoting substances as well the mycorrhizal fungi increased the availability of most nutrient elements specially phosphorus and micro-nutrients which encourage the growth of different soil micro-organisms **(Bellone and de Bellone, 1993)**.

As regard to the effect of viral and mycorrhizal inoculation on fungi counts data in Fig. (2) show that, rhizosphere of untreated plants contained higher populations of fungi than the rhizosphere of treated plants with virus or/and VAM fungus. The mycorrhizal or viral inoculation decreased the fungi counts and the combination of BCMV and *G. macrocarpum* led to sharp decrease in soil fungi populations and this was true at all growth periods. This indicate that the substances of root exudates of the viral infected plants may contains some fungal inhibitors **(Abd El-Mageed, 1992)**. In addition, **Eisa (Nawal) *et al.* (1994)** reported that, the mycorrhizal fungi decrease or inhibit soil-borne fungal diseases.

Effect of Viral, Fungal and VA-Mycorrhizal Inoculation on Bean Growth Characters :

Data in Table (2) emphasize that, VAM fungus gave highly increase in plant height when inoculated alone as well as when associated either with *R. solani* or slightly with BCMV compared with the control (1) in sterilized soil. The same trend (with VAM) was observed under non-sterilized soil and these results indicated that, harmful effect of the tested pathogens (*R. solani* or BCMV) was more decreased when it was combined with VA-Mycorrhiza and this trend agreed with results obtained by **Blaszkowski (1993)** who stated that, wheat plants inoculated with mycorrhizal fungi were significantly taller than uninoculated one. In contrast, plant height significantly decreased due to inoculation with *R. solani* or BCMV each one alone, while, in case of control (1), fungal and viral infection and fungal, viral and mycorrhizal inoculation gave the same values (68.33 cm) of plant height. Also, data show that, compared with the control, in all treatments the number of leaves/plant were increased with the exception of both *R. solani* and BCMV treatments when inoculated alone regardless the type of soil, and the same number of leaves/plant was obtained with *R. solani* and BCMV when combined with VAM fungus and it increased than with each pathogen alone. This result clearly indicated an antagonistic reaction between VAM fungus and *R. solani* or BCMV which may inhibit the harmful effect of each pathogen alone. Similar results were obtained in case of viral infection by **Fawzy (1973), Rizkalla (1977) and Fawzy and Abd El-Mageed (1990)**. As regard to fresh weight of root system, inoculation of soil at sowing with mycorrhizal fungi gave the highest values of fresh root system weight and this was obvious in sterilized soil, while, all treatments significantly decreased fresh weight of root system in sterilized or non-sterilized soil except *R. solani* + BCMV treatment which slightly increased the fresh weight of root system. Compared with the control (2) data show that, dry weight of root system decreased in case of non-sterilized soil treatments, except in case of VAM fungus. Treatments of *R. solani* and BCMV either each one alone or together in sterilized soil showed decrease in dry weights of roots compared with the control (1) treatment. This reduction was obvious specially in case of treatments included BCMV regardless the type of soil, while, opposite result was recorded with VAM fungus treatments in sterilized soil. Also, obtained data in Table (2) show that, fresh and dry weights of shoot system compared with the control in sterilized soil increased in all treatments including VAM fungus except when it was associated with the combination of *R. solani* and BCMV altogether. Also, the weight of shoot system was decreased in case of *R. solani* or BCMV when each one was used alone, while, their combination led to increase in fresh and dry weights of shoot system. Many investigators reported that, several viruses decrease the activity of fungal infection **(Zink and Duffus, 1975; Allam *et al.*, 1978; El-Hammady *et al.*, 1983 and Fawzy and Abd El-Mageed, 1992)**. While, the others reported that, several fungi inhibit the infectivity of viral infection **(Gessler and Kuc, 1982 and Abd El-Mageed, 1995)**.

As regard to non-sterilized soil, the fresh and dry weight of shoots was increased in the VAM fungus treatments compared with the other treatments, also, data show that, fresh and dry weight of shoots were decreased in most treatments compared with the control (2).

This could be attributed to the effect of virus on different physiological processes in plant which lead to stunting of the vegetative parts of the plant while, their combined (viral and fungal infection) caused considerable increase in shoots (fresh and dry weights) this, could be attributed to an antagonistic effect between the fungal metabolites and virus (**Abd El-Mageed, 1986**). While, **Amer *et al.* (1983)** recorded that, single and double infections with BCMV and *Myrothecium verrucaria* markedly decreased the dry weight of bean leaves.

As regard to number of flowers/plant data showed that, regardless the type of soil, BCMV treatment alone also *R. solani* as alone or with VAM fungus decreased the number of flowers compared with the control. In this respect, viral infection has been reported to reduce the number of flowers of diseased plants (**Goth and Wilcoxson, 1962 and Allam, 1965**) and caused shedding of the flowers and pods. On the contrary, VAM fungus and all treatments combined with it almostly increased the number of flowers/plant except when combined with *R. solani*. This may be due to that mycorrhizal plants absorbed more phosphorus and the percentage of phosphorus was significantly higher in the roots and tops of mycorrhizal plants than in the non-mycorrhizal ones (**Gerdemann, 1964 and Holeuas, 1966**).

Effect of Viral, Fungal and VA-Mycorrhizal Inoculation on Some Chemical constituents of Bean Plants :

Data in Table (3) indicate that, compared with the control total carbohydrates increased in most of treatments specially in case of treatments i.e VAM fungus or *R. solani*, each one alone and the combination of BCMV with VAM fungus also the treatment included all of them and this was true in both investigated soils. On the other hand, in case of infection with BCMV carbohydrate reduction may be classified into three categories : severe reduction in plants inoculated with BCMV alone or with *R. solani* together in sterilized soil and intermediate reduction in plants inoculated with BCMV in non-sterilized soil, while, the slight reduction was in plants inoculated with the combination of *R. solani* and VAM fungus treatment. In this respect, **Gamal El-Din *et al.*, (1990)** found that, total carbohydrates percentage of the leaves was increased in case of the infection with *F. moniliforme* in bean plants. On the other hand, **Amer *et al.* (1983)** reported that, single infection with BCMV or double infection of bean plants with virus and *Myrothecium verrucaria* decreased the total carbohydrates content.

As regard to pigments compared with control (1) chlorophyll A increased in all treatments except in case of plants inoculated with BCMV alone in sterilized soil and the chlorophyll A level almostly proportionated with carbohydrates except in case of BCMV regardless the type of soil. While, chlorophyll B was increased with all treatments in sterilized soil except in plants inoculated with BCMV and the combination of BCMV + *R. solani* + VAM fungus. As well as chlorophyll B was

increased under all treatments in non-sterilized soil except for BCMV treatment compared with the control (2).

On the other hand, data show that, chlorophyll C increased with all treatments except in case of plants inoculated with BCMV + VAM fungus in non-sterilized soil. However, **Mahdy (1981)** found that, all determined pigments recorded lower values due to infestation with *F. oxysporum* f.sp. *vasinfectum* in some cotton varieties. While, **Rizkalla (1983)** recorded that, broad bean wilt and bean yellow mosaic viruses reduced the three pigments in infected Giza 1 and 2 samples collected at different intervals of infection.

Also, data in Table (3) indicate that, compared with the control total nitrogen and protein increased in all treatments specially in plants grown in soil inoculated with VAM fungus combined with BCMV and *R. solani* , while, the lowest percentage of total protein was observed in case of viral inoculation alone although still higher than control healthy plants. The same results were obtained with respect to the total phosphorus, while, total potassium almostly increased in all treatments specially in case of those including BCMV or VAM fungus alone regardless the type of soil.

As regard to N, P and K, **Fawzy (1973)** found that, infection of broad bean plants with PMV and BBMV raised the total nitrogen content of leaves while, the decreased in stem. Whereas, total phosphorus content increased in leaves and stems while, infection of broad bean plants with PMV decreased the total potassium content and BBMV infection increased the potassium in leaves and decreased it in stem. **Amer *et al.* (1983)** reported that, virus infection (BCMV) increased total nitrogen content of inoculated leaves above healthy ones followed by combined inoculation (BCMV + *Myrothecium verrucaria*). On the contrary, fungal inoculation diminished greatly total nitrogen percentage. While, **Rizkalla (1983)** mentioned that, a reduction in phosphorus ranging from 5.7 to 26.1% and from 1.7 to 28% was observed in Giza 1 broad bean plants infected by BBMV or BYMV, respectively, while, the amount of phosphorus increased in Giza 2 bean with virus infection.

As regard to K, **Fawzy and Abd El-Mageed (1990)** studied the effect of infection with combinations of fungi *F. moniliforme* and *T. roseum* and viruses BCMV and BYMV on K contents of bean plants and reported that, all of the applied treatments increased the percentage of potassium with the exception of in case BCMV + *F. moniliforme* + *T. roseum* which decreased the K percentage.

Table (3) : Effect of viral, fungal and VA-Mycorrhizal inoculation on some chemical contents of bean plants.

Parameters / Treatments	Total tea mg/g dry matter	Chlorophyll (mg/g) fresh matter A	B	C	Protein content (%)	Total Nitrogen (%)	Total Phosphorus (%)	Total Potassium (%)
A-Sterilized soil								
Control (1)	22.5	1.80	1.34	0.495	5.46	0.875	0.302	2.12
R. solani	47.5	2.43	1.42	1.11	10.75	3.00	0.770	2.81
Bean common mosaic (BCMV)	7.50	1.07	0.91	1.15	10.93	1.75	0.375	3.40
G. macrocarpum	50.0	2.47	1.708	0.906	24.56	3.93	0.425	4.37
R. solani + BCMV	12.5	2.08	1.52	0.758	15.62	2.50	0.620	3.10
R. solani + *G. Macro.*	22.0	1.94	1.43	0.855	27.31	4.37	0.992	4.30
G. macro. + BCMV	47.0	1.837	1.37	0.54	17.18	2.75	0.312	2.60
R. solani+BCMV + *G. macro.*	45.0	2.13	0.799	0.729	32.00	5.12	0.530	3.10
B-Non-sterilized soil								
Control (2)	35.0	2.61	0.798	1.020	9.37	1.50	0.385	1.62
BCMV	17.5	2.33	0.777	0.821	15.62	2.50	0.375	2.65
G. macrocarpum	42.5	2.17	1.008	0.801	21.87	3.50	0.695	3.00
G.macrocarpum + BCMV	43.0	2.23	0.871	0.423	16.12	2.90	0.856	2.40

CONCLUSION

From the obtained results, it can be concluded that, the inoculation with mycorrhizal fungi should be applied at sowing time to decrease the infection with root-rot and damping-off diseases and minimize the harmful effect of viral diseases as well as mycorrhizal inoculation improve the plant growth since the VAM fungus increase both carbohydrates and protein content as well nutritional elements in the plants

REFERENCES

Abdel Kadir, N. E. (1977). Pathological and histological studies on Hemp root-rot disease and its control. M. Sc. Thesis, Fac. Agric., Cairo Univ., Egypt.

Abd El-Mageed, M. H. (1981). Studies on some diseases that attack the roots of *Phaseolus vulgaris* plant. M. Sc. Thesis, Fac. Agric., Zagazig Univ., Egypt.

Abd El-Mageed, M. H. (1986). Interaction between infection of *Phaseolus vulgaris* plant with *Trichothecium roseum* and *Fusarium moniliforme* and some important viral disease in A. R. E. Ph. D. Thesis, Fac. Agric. Moshtohor, Zagazig Univ., Benha Branch.

Abd El-Mageed, M. H. (1992). Interaction between virus infection and fungal diseases in bean plants (*Phaseolus vulgaris* L.) I- Studies on PG-inhibitor from root and root exudates. Annals of Agric. Sci. Moshtohor, 30(3): 1233-1246.

Abd El-Mageed, M. H. (1995). Inhibitory effect of some fungal growth products on Bean Common Mosaic Virus infectivity and seed germination of BCMV-infected bean plants (*Phaseolus vulgaris* L.) . Annals of Agric. Sci. Moshtohor, 33(4): 1291-1305.

Al-Fassi, F. A.; Abo-Zinada, R. A.; Malibari, A. A.; and Ramadan, E. M. (1990). Effect of inoculation with Vesicular-Arbuscular-Mycorrhiza on plant growth. Ann. Agric. Sci., Fac. Agric. Ain-Shams Univ., 35(1):125-142.

Allam, E. K. (1965). A study of squash mosaic virus disease. Plant Dis. Reptr., 49 :218-221.

Allam, E. K.; Ali, M. D. H.; and Abo El-Ghar, A. (1978). The mode of inhibitory action and translocation of some fungal growth products on Tobacco Mosaic Virus and Cucumber Mosaic Virus infectivity. Proceeding of 4th Conf. Pest Control NRC., Cairo (1978)

Allen, O. N. (1950). Experiments on soil bacteriology. Burgess Publishing Co. Minnesota, U.S.A.

Amer, M. A.;El-Hammady, M. ; and Abo El-Abbas, F. (1983). Effect of single and double infection with *Myrothecium verrucaria* and common bean mosaic virus (CMBV) on dry weight and chemical constituents of bean plants. Acta Phytopathologica, Academiae Scientiarum, Hungaricae, 18(4): 225-235.

American Public Health Association (1989). Standard methods for the examination for water and waste water. Washington, D. C., U.S.A.

Anon., (1995). Agricultural Economic Report, 1994 (In Arabic).

A.O.A.C.(1975). Official Methods of "Analysis of Association of Official Agricultural Chemists. 13th Ed., Washington, D. C., U.S.A.

Bellone, C. H. and de Bellone, S. D. V. (1993). Nitrogenase, cellulase and pectinase activity in sugar cane roots inoculated together with VA-Mycorrhiza and nitrogen fixing bacteria. Proc. of 6th Inter. Sym. on N2-fixation with non legumes. Ismailia, Egypt, 6-10 September, 69-75.

Beniwal, S. P. S. and Gudawskas, R. T. (1974). Maize dwarf mosaic virus increase susceptibility of sorghum and corn to *Helminthosporium maydis* race T. Phytopathology, 64:1197-1201

Blaszkowski, J. (1993). Effect of five *Glomus* spp. (Zygomycetes) on growth and mineral nutrition of *Triticum aestivum* L. Acta Mycologica, 28(2): 201-210.

Brown, L. (1959). Phytopathological work during 1958 compiagum in bean growing. Estac. Agric. Lanolina, 33(338): 1-10. (c. f. Rev. Appl. Mycol., 1962:74).

Brown, J. B. and Lilliland, L. I. (1946). Rapid determination of potassium and sodium in plant material and soil extract by flame photometer. Proc. Amer. Soc. Hort. Sci., 48:301-346.

Daft, M. J. and Okussanya, B. O. (1973). Effect of endogone mycorrhiza on plant growth V: Influence of infection on the multiplication of viruses in tomato, petunia and strawberry. Phytopath., 63:975-983.

Davis, R. M. and Menge, J. A. (1980). Influence of *Glomus fasiculatus* and soil phosphorus on *Phytophthora* root-rot of citrus. Phytopathology, 70:447-452.

Deif, A. A. (1977). Studies on a seed-borne-bean common mosaic virus. M. Sc. Thesis, Fac. Agric., Tanta Univ., Egypt.

Dixon, G. R. (1981). A Text Book of Vegetable Crop Diseases. ISBN 0-333-23574-6 London (UK)- Macmillan Pub. 1981-404.

Eisa, Nawal A.; Ahmed, K. G. M.; Mahdy, A. M. M.; Badr, A. E.; and Abdel-Latif, Faten M. (1994). Effect of seed treatment with fungicides combined with VA-Mycorrhiza on plant growth and root-rot diseases of broad bean (*Vicia faba* L.) . Egypt. J. Appl. Sci., 9(11): 375-395.

El-Deepah, H.R.A. (1981). Efficiency of vesicular arbuscular mycorrhiza in seven crop species modified by NaCl in the soil. Ph. D. Thesis Gottingen Univ., F.R. of Germany.

El-Hammady, M.; Elewa, I. S.; Mostafa, M. H.; and Abo El-Abbas, F. (1983). Interaction between fungal disease and virus infection . II. *Myrothecium verrucaria* Ditmar and bean common mosaic virus in bean plants special reference to the effect of fungus-toxins on TMV-infection. J. Agric. Res., Tanta Univ. 9(1).

Evans, T. A. and Stephens, C. T. (1989). Increased susceptibility to *Fusarium* crown and root-rot in virus-infected asparagus. Phytopathology, 79: 253-258.

Fawzy, R. N. (1973). Studies on broad bean virus diseases in Egypt. M. Sc. Thesis, Fac. Agric., Cairo Univ.

Fawzy, R. N. and Abd El-Mageed, M. H. (1990). Response of bean plants to artificial infection with different combinations of fungi and viruses. Ann. Agric. Sci., Fac. Agric. Ain-Shams Univ., Cairo, Egypt., 35(1): 393-405.

Gamal El-Din, I. F.; Mahdy, A. M. M.; and Abd El-Mageed, M. H. (1990). Effect of some fungicides on interaction between fungal and viral infection in bean (*Phaseolus vulgaris* L.). Egyptian J. Food Sci., 18 (1-3): 267-276.

Garcia-Garrido, J. M. and Ocampo, J. A. (1987). Interactions between VAM and plant pathogenic organisms. Anales de Edafologia y Agrobiologia, 46(9/10) 1223-1245. (c. f. Rev. Pl. Path., 67(12):5748).

Gerdemann, J. W. (1964). The effect of mycorrhiza on the growth of maize. Mycologia, 56:342-349.

Gessler, C. and Kuc, J. (1982). Induction of resistance to ***Fusarium*** wilt in cucumber by root and foliar pathogens. Phyto., 72:1439-1441

Goth, R. W. and Wilcoxson, R. C. (1962). Effect of bean yellow mosaic virus on survival and flower formation in red clover. Crop Sci., 2:426-429. (c. f. Rev Appl. Mycol., 42:201).

Gowily, Ahlam M. (1987). Effect of soil and irrigation on damping-off disease in some leguminous plants. Ph D. Thesis, Dept. Agric. Botany, Fac. Agric., Zagazig Univ.

Holcuas, C. D. (1966). The effect of vesicular-arbuscular-mycorrhiza on the uptake of soil phosphorus by strawberry (*Fragria* sp. var. Combridge Favourite). J. Hort. Sci., 41:57-64.

Jayashree, J. ; Dinesh, K.; Jayaraman, J.; and Kumar, D. (1995). Influence of mungbean yellow mosaic virus on mycorrhizal fungi associated with *Vigna radiata* var. PS16. Indian Phytopathology, 85(1):108-110.

Khan, I. D. (1966). Saprophytic behaviour, inoculum potential and infection of cotton root-rot infection fungi. Ph. D. Thesis, Fac. Agric., Cairo Univ., Egypt.

Kope, H. H. and Fortin, J. A. (1990). Anti-fungal activity in culture filtrates of the ectomycorrhizal fungus *Pisolithus tinctorius*. Canadian J. of Botany, 68(6): 1254-1259.

Magyarosy, A. C. and Hancock, J. G. (1974). Association of virus induced changes in laimosphere microflora and hypocotyle exudation with production to *Fusarium* stem rot. Phytopathology, 64:994-1000.

Mahdy, A. M. M. (1981). Physiological studies on some cotton varieties infected with *F. oxysporum* f.sp. *vasinfectum*. M. Sc. Thesis, Fac. Agric., Moshtohor, Zagazig Univ. (Benha Branch).

Martin, J. P. (1950). Use of acid rosebengal and streptomycin in the plate method for estimating soil fungi. Soil Science, 69(3): 215.

Michel, K. A. ; Gilles, J. K.; Ramilton, R. P. A.; and Smith, F. (1956). Colourimetric method for determination of sugars and related substances Anal. Chem., 28:3.

Omar, R. A.; El-Khadem, M. ; and Dief, A. A. (1982). Studies on seed-borne bean common mosaic virus. I- Identification of the virus. III- Effect of the virus on biological yield and chemical characters of bean seeds. Rev. Pl Path., 61: 4449-4451.

Omer, S. A. M. (1986). Pathological studies on root-rot disease of faba bean (*Vicia faba* L.). FABIS Newsletter, No. 14: 34-37

Philips, J. M. and Hyman, D. S. (1970). Improved features for clearing roots and staining parasitic and vesicular-arbuscular-mycorrhizal fungi for rapid assessment of infection. Trans. Br Mycol. Soci., 55:158-161.

Piper, G. S. (1947). Soil and Plant Analysis The Univ. of Adrlaide.

Pregl, F. (1945). Quantitative organic micro-analysis. 4th. Ed. J. and A. Churchill, Ltd., London.

Reyes, A. A. and Chadna, K. C. (1972). Interaction between *Fusarium oxysporum* f.sp. *conglutinants* and turnip mosaic virus in *Brassica campestris* var. *chinensis* seedlings. Phytopathology, 62:14-24.

Rizkalla, L. R. (1977). Studies on some viruses affecting broad bean in Egypt. M. Sc. Thesis, Fac. Agric., Ain-Shams Univ., Cairo, Egypt.

Rizkalla, L. R. (1983). Physiological effects of some broad bean viruses. Ph. D. Thesis, Fac. Agric., Ain-Shams Univ., Cairo, Egypt.

Salt, G. A. (1982). Factors affecting resistance to root-rot and wilt diseases. Faba bean improvment. edited by G. Hertin and C. Webb, pp. 259 (c. f. Rev. Pl. Path., 26(10): 4536).

Schnobeck, F. (1987). Mycorrhiza and plant health. A contribution to biological protection of plants. Angewanddte Botanik, 61(1/2):9. (c. f. Rev. Pl. Path., 66:4093).

Schnobeck, F. and Schinzer, U. (1972). Investigation on the influence of endotrophic mycorrhiza on TMV lesion formation in *Nicotiana tabacum* L. var. Xanthinc. Phytopathology, 62:78-80.

Skinner, F. A.; Jones, P. G.; and Mollison, J. E. (1952). A comparison of direct and plate counting technique for quantitative counts of soil microorganisms. J. Gen. Microbiol., 6:261-271.

Snedecor, G. W. and Cochran, W. G. (1982). Statistical Methods. 6th. Ed. Iowa. State Univ. Press, Iowa, U. S. A.

Wettstein, D. (1957). Chlorophyll-lethal und der submikroskopische formwechsei der plastidem. Exptl.Cell Res., 12:427-506. (English summary)

Whithead, M. D. (1975). Sorghum grain, a medium suitable for the increase of inoculum for studies of soil-borne and certain other fungi. Phytopathology, 47:450.

Zamblin, L. and Schenck, N. C. (1983). Reduction of the effects of pathogenic root-infecting fungi on soybean by the mycorrhizal fungus *Glomus mosseae*. Phytopathology, 73: 1403-1483.

Zink, F. W. and Duffus, J. E. (1975). Reaction of turnip mosaic virus susceptibility and downy mildew (*Bremia lactuca*) resistance in lettuce. Phytopathology, 65: 243-245.

التفاعل بين الإصابة الفيروسية والأمراض الفطرية على نباتات الفاصوليا

٢- كفاءة التلقيح بالميكورهيزا على فعالية الإصابة بكل من فيروس موزايك الفاصوليا العادي وفطر الريزوكتونيا سولاني

محمد هرون عبد المجيد — راشد عبد الفتاح زغلول

فرع الفطـــر وأمراض النبات — فرع الميكروبيولوجي

قسم النبات الزراعي - كلية الزراعة بمشتهر - جامعة الزقازيق - مصر

عند دراسة تأثير التلقيح بفطر الميكورهيزا (*Glomus macrocarpum*) على فعالية الإصابة بفيروس BCMV وفطر *R. solani* على نباتات الفاصوليا (صنف جيزة ٣) أوضحت النتائج مايلي :-

أن التلقيح بفطر *R. solani* أعطى أعلى نسبة من مرضـي سقوط البادرات فوق سطح التربة Post emergence damping-off وعفن الجذور Root-rot بينما قلت هذه النسبة عند المعاملة بفطر الميكورهيزا وفيروس BCMV كل على حده.

وكانت أقل نسبة لكلا المرضين عند التلقيح المزدوج بكل من فطر الميكورهيزا والفيروس.

أعلى نسبة للإصابة الفيروسية حدثت عند التلقيح بفطر الميكورهيزا بينما أدى التلقيح بفطر *R. solani* إلى نقص النسبة المئوية للنباتات المصابة بالفيروس ، وعند التلقيح المزدوج بكل من فطر الميكورهيزا وفطر *R. solani* أدى ذلك إلى ظهور أعلى نسبة نقص للنباتات المصابة بالفيروس وذلك في التربة المعقمة .

أدى الحقن بالفيروس إلى نقص كثافة المستعمرات وإنتاج الجراثيم لفطر الميكورهيزا بجذور الفاصوليا بغض النظر عن نوع التربة وكان أعلى نقص في حالة التلقيح بفطر *R. solani* أو الفيروس أو كلاهما معاً.

عند التلقيح المزدوج بفطر الميكورهيزا والحقن بفيروس BCMV أدى ذلك إلى زيادة العدد الكلي لبكتيريا التربة في منطقة الريزوسفير في كل فترات النمو ، وكذلك أدى التلقيح بفطر الميكورهيزا سواء منفرداً أو مع الحقن بفيروس BCMV إلى زيادة عدد الأكتينوميسيتات في التربة . وعلى العكس من ذلك فقد وجد أن الحقن بالفيروس أوالتلقيح بفطر الميكورهيزا أدى إلى نقص أعداد الفطريات بالتربة وخصوصاً عند التلقيح المزدوج بهما حيث أدى ذلك إلى نقص حاد في أعداد الفطريات في التربة .

أدى التلقيح بفطر الميكورهيزا إلى زيادة معنوية في طول النباتات ، عدد الأوراق / نبات ، الوزن الطازج والجاف للمجموع الجذري والخضري ، عدد الأزهار / نبات سواء عند التلقيح به منفرداً أو عند تلقيحه مع فطر *R. solani* أوحقنه بفيروس BCMV بغض النظر عن نوع التربة ، بينما حدث نقص في كل صفات النمو الموضحة سابقاً عند الحقن بفيروس BCMV أو فطر *R. solani* وذلك في التربة المعقمة والغير معقمة.

وقد أوضحت النتائج ان محتوى المجموع الخضري من الكربوهيدرات والبروتين الكلي وعناصر النيتروجين والفوسفور والبوتاسيوم زاد في جميع المعاملات الملقحة بفطر الميكورهيزا.

وكانت نسبة كلوروفيل A متناسبة مع المحتوى الكربوهيدراتي ماعدا التلقيح بالفيروس ،وأدى التلقيح بفطر الميكورهيزا إلى زيادة محتوى النباتات من كلوروفيل B ، بينما كلوروفيل C زاد مع كل المعاملات ماعدا معاملة الحقن بالفيروس والتلقيح بفطر الميكورهيزا في التربة الغير معقمة .

ومن نتائج هذا البحث ينصح بإجراء التلقيح بفطريات الميكورهيزا للحد من الإصابة بأمراض سقوط البادرات وأعفان الجذور وتقليل التأثير الضار للأمراض الفيروسية ولتحسين حالة نمو النبات حيث اتضح أن فطريات الميكورهيزا تعمل على زيادة محتوى النباتات من الكربوهيدرات والبروتين وكذلك العناصر الغذائية.