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INTERACTION EFFECT OF RHIZOBIAL INOCULATION ON VIRAL AND FUNGAL INFECTION IN BROAD BEAN (*Vicia faba* L.)

BY

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ABSTRACT

The interaction effect of inoculation of broad bean seeds with proper strain of *Rhizobium leguminosarum* biovar *viceae*, on damping-off and root-rot diseases caused by *Rhizoctonia solani* and viral infection by bean common mosaic virus (BCMV) was studied under sterilized and non-sterilized soil.

Data showed that *R. solani* when infested solely in sterilized soil caused the highest percentage of post-emergence damping-off and root-rot disease. While, rhizobial and viral inoculation reduced of studied fungal infection either when used solely or in combination. Highest percentage of viral infected plants was observed with viral inoculation solely whereas, viral infected plants were decreased in other treatments specially in case of rhizobial inoculation in both investigated soils. The highest values of fresh and dry weights of root nodules were recorded with rhizobial inoculation treatment whereas, root nodules weights were highly decreased when *R. solani* or BCMV were inoculated with *Rh. leguminosarum* either solely or in combination.

Total bacterial counts and actinomycetes were high in rhizosphere of rhizobial inoculated plants either solely or in combination with viral infection and this trend was observed at all growth stages. Rhizosphere of inoculated plants with *Rh. leguminosarum* and/or BCMV contained lower populations of fungi than that of uninoculated plants.

Rhizobial inoculation treatments gave significant increase in plant height, no. of leaves/plant, no. of flowers/plant, fresh and dry weights of root and shoot system when inoculated solely as well as when associated either with *R. solani* or BCMV regardless the type of soil. On the contrary, all growth characters were decreased in case of viral or fungal infection each one solely, but increased with their combination regardless the type of soil.

Also, total nitrogen and crude protein were increased with rhizobial inoculation either solely or combined with *R. solani* or BCMV. Except the control treatment, the lowest value of total nitrogen and crude protein was observed with fungal infestation solely in sterilized soil. Total phosphorus was the highest in case of fungus combined with virus or Rhizobium combined with virus treatments in sterilized and non-sterilized soil, respectively. While, the lowest value of total phosphorus was obtained with fungal infestation solely in sterilized soil.

Chlorophyll a, b and c greatly increased with rhizobial inoculation either solely or in combination with *R. solani* or BCMV regardless the type of soil. Except the control, chlorophyll a, b and c levels were the lowest in case of *R. solani*, BCMV or *R. solani* + BCMV, respectively, in sterilized soil. While, chlorophyll b or c in case of viral inoculation solely was lower than other treatments in non-sterilized soil.

Total carbohydrates level was the highest with rhizobial inoculation solely in both soils, while, the lowest level of total carbohydrates was observed with *R. solani*, BCMV each one solely in sterilized and non-sterilized soil, respectively. On the other hand, rhizobial inoculation in sterilized soil combined with either *R. solani* or BCMV showed increase in carbohydrates level compared with inoculation with either *R. solani* or BCMV each one solely.

INTRODUCTION

Vicia faba L. is one of the most important leguminous crops in Egypt. area of broad (faba) bean has increased to 342168 feddan in 1994 (Annon., 1995). The most common and economically important pathogen causing damping-off and root-rot diseases on broad bean is *R. solani* (Yehia and Hassan. 1982; Nofal *et al.*, 1982; Gowily (Ahlam), 1987 and Eisa (Nawal) *et al.*, 1994).

Bean common mosaic virus (BCMV) as a potyvirus was reported to infect several legume crops (*Phaseolus vulgaris, Vicia faba, Lupinus alba, Pisum sativum* and *Trifolium pratense*) by Bos (1971), Ordosgoitty (1972), Meiners *et al.*, (1978) and Sanudo and Galvez (1979). Also, a strain of bean common mosaic potyvirus infect soybean in India was identified on the basis of serological tests, transmission, host range, physiological and biological characteristics by Ghosh and Dhingra (1993). On the other hand, Al-Shahwan and Abdalla (1991) observed severe mosaic in a broad bean field in Saudi Arabia and identified bean common mosaic virus on the basis of host-range, physical properties, virus morphology and serology.

Under field conditions crops may be infected with more than one pathogen (Dixon, 1981) and the combination between these pathogens may show synergistic effect (Reyes and Chadna, 1972 and Stevens and Gudauskas, 1983) or antagonistic effect (Magyarosy and Hancock, 1974; El-Hammady *et al.*, 1983; Abd El-Mageed, 1986; Gamal El-Din *et al.*, 1990 and Abd El-Mageed, 1992).

Many workers studied the relation between legume crops and rhizobial inoculation (Hamdi, 1976; Kremer and Patterson, 1983; Abdel-Nasser *et al.*, 1988; Zahra *et al.*, 1990 and Gohar *et al.*, 1991). They reported that rhizobial inoculation significantly increased the plant height, no. of flowers, plant dry weight, N-content, dry weight of nodules and yield of faba bean, chick pea and soybean plants. The relation between rhizobial inoculation and viral infection in some legume crops was studied by Singh and Mall (1974), Mali *et al.*, (1977) and Fugro and Mishra (1993). As well, the relation between fungal infection and rhizobial inoculation was studied by several investigators (Bondara, 1978; Bharagova *et al.*, 1979 and Patil, 1985). So, the present work was carried out to study the interaction between viral (BCMV) and/or fungal (*R. solani*) infections of broad bean when seeds inoculated with specific and effective strain of *Rhizobium*.

MATERIALS AND METHODS

Source of pathogenic agents :

1- The fungus :

Two isolates of *Rhizoctonia solani* Kuhn were isolated from naturally infected broad bean seedlings showing damping-off and root-rot and collected from Farm of Fac. Agric., Moshtohor, Zagazig Univ. Purification of the isolated fungi was carried out using hyphal tip technique and identified according to Parameter and Whitney (1970) and also in Plant Pathology Institute, Agric. Res. Center, Giza, Egypt.

2- The virus :

Bean common mosaic virus (BCMV) was obtained from naturally infected bean plants (*Phaseolus vulgaris* L.) and identified according to the host range, differential hosts, transmission and physical properties (Abd El-Mageed, 1986) and continually maintained in a freezing infected leaves collected from recently inoculated plants.

Source of rhizobial strain :

An effective strain (*Rhizobium leguminosarum* biovar *viceae*) was obtained from Agric. Res. Center, Water and Soil Res. Inst, Dept. of Microbiology, Giza, Egypt.

Estimation of fungal isolates conformity and inoculum potential :

The inoculum of each fungal isolate was grown on sterilized sand sorghum grain medium (Whithead, 1975). Clay pots (No. 30) were sterilized properly using 5% formalin solution. A clay soil was autoclaved at 15 lb/in.^{2} for 3 hr and infested with different amount of inoculum *i.e.* 0.5, 1.0, 3.0, 5.0 and 8.0% of soil weight. Ten broad bean seeds of Giza-2 cv. were sown in each pot in four replicates and kept under insect-proof greenhouse. Post-emergence damping-off and the percentages of root-rot (after Salt, 1982) were recorded (15 - 45) and (60) days from sowing, respectively.

The aggressive isolate and potential inoculum rate were chosen according to obtained results and subjected to all experiments carried out in this study.

Fungal infestation :

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

Rhizobial inoculum preparation :

Preparationof inoculum was carried out according to Gohar *et al.*, (1991). 7-days-old rhizobial culture grown on yeast mannitol agar medium was suspended in sterile water to obtain a homogenous population of 2 x 10^8 cell/ml. The carrier used was peat containing $CaCO_3$ (5% w/w) as a neutralizing material, sterilized at 120°C for 4 hr, and thoroughly mixed with rhizobial suspension at the rate of 2:1 (rhizobial suspension: Peat) after cooling. Seeds were mixed with suitable amount of arabic gum, then thoroughly mixed with the carrier containing the specific rhizobial strain to ensure sufficient coating by inoculum. This process was carried out just before planting.

Cultivation process :

Ten broad bean seeds Giza-2 cv. were sown (10th Oct., 1994) at a depth of nearly 2cm 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 analyses determinations, while, the others were used in periodical analyses i.e. pathological and microbiological determinations. All pots had been supplied with the equal amounts of N and P_2O_5 as ammonium sulphate and super-phosphate at a rate of 15 and 30 kg/fed., respectively, in two equal doses at vegetative and flowering stages.

Viral inoculation after two weeks of sowing :

The seedlings 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 25-days-old infected beans leaves (*Phaseolus vulgaris* L.). Infested leaves were rinsed with tap water.

Combinations of fungal infestation, viral inoculation and rhizobial inoculation in sterilized and non-sterilized soils were designed as follows:

A- Sterilized soil :

- Control (1). - Infection with *R. solani* only.
- Inoculation with (BCMV) only.
- Inoculation with *Rhizobium leguminosarum* biovar *viceae* only.
- *R. solani* + BCMV. - *R. solani* + *Rh. leguminosarum*.

- BCMV + *Rh. leguminosarum*. - *R. solani* + BCMV + *Rh. leguminosarum*.

B- Non-sterilized soil :

- Control (2).
- Inoculation with *Rh. leguminosarum* biovar *viceae* only.
- Inoculation with bean common mosaic virus (BCMV) only.
- BCMV + *Rh. leguminosarum*.

The determinations :

A- Disease assessment :

1- Percentage of post-emergence damping-off 45-days-old.

$$\% \text{ Post -emergemce damping -off} = \frac{\text{number of dead seedlings}}{\text{total number of seedlings}} \times 100$$

2- Root-rot disease of plants. 60-days-old plants were carefully removed, washed thoroughly with tap water and examined for 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, (1982).

3- Percentage of viral infected plants.

$$\% \text{ Viral infected plants} = \frac{\text{number of virus infected plants seedlings}}{\text{total number of inoculated plants}} \times 100$$

B- Microbiological determinations :

Total microbial count, Actinomycetes and fungi were counted in broad bean rhizosphere in non-sterilized soil treatments only. The rhizosphere of soil samples were collected at vegetative, tillering, flowering and maturity stages. The soil extract yeast agar medium was used for counting the total microbial flora (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 fungi. The plate count method was used for the three determinations.

C- Growth characters :

- Plant height (cm).
- Leaves number/plant.
- Flower number/plant.
- Fresh and dry weights of root system (g/plant).
- Fresh and dry weights of shoot system (g/plant).
- Fresh and dry weights of nodules (g/plant) for rhizobial inoculated and non-sterilized soil treatments.

D- Chemical analysis :

1- 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:

Crude protein = total nitrogen x 6.25 (A.O.A.C., 1975)

2- Total phosphorus was determined in the dry matter of shoot system colourimetrically according to American Public Health Association (1989).

3- Chlorophyll a, b and c were estimated in the 3rd leaf of the plant according to Wettstein, (1957).

4- 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.

Statistical Analysis:

All data presented in percentages such as disease assessments were transformed to arcsin and subjected as well as data of growth characters to analysis of variance according to Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Pathogenicity and inoculum potential of *R. solani* isolates on broad bean plants :

Data in Fig. (1) showed that, the isolate (I) was more aggressive than the isolate (II). The same figure also clearly showed that the percentage of post-damping-off and root-rot diseases were increased with increasing the inoculum potential of both *R. solani* isolates. This is in agreement with many earlier investigators, Khan (1966), Abd El-Kadir (1977) and Omer (1986) who mentioned that, on the basis of pathogenesis, *R. solani* was the most virulent in causing post-emergence phase and root-rot disease and these diseases were increased with increasing the inoculum potential. So, the isolate I was chosen to carry out the further experiments in this study.

Effect of rhizobial, viral inoculation and fungal infestation on disease severity and nodulation :

Data in Table (1) showed that post-emergence damping-off and disease severity index (DSI) of root-rot were higher in sterilized soil due to infestation with *R. solani* solely than in all other treatments in both sterilized and non-sterilized soils. These results are in agreement with many investigators (Mahdy, 1985; Omer, 1986; Gowily (Ahlam), 1987 and Eisa (Nawal) *et al.*, 1994).

Damping-off and DSI caused by *R. solani* in sterilized soil were reduced by using either *Rhizobium leguminosarum* or BCMV and both of them. The same trend was observed in non-sterilized soil.

Regarding to rhizobial inoculation, Tu (1980) reported that, seed inoculation with root nodulating bacteria before planting decreased post-emergence damping-off and root-rot diseases caused by *F. oxysporum* and increased the total N-content. Chakraborty and Chakraborty (1989) found that, seed bacterization with *Rh. leguminosarum* biovar *viceae* was highly effective in reducing the severity of root-rot of pea. Also, the same trend was observed by Ehteshamul-Haque and Ghaffar (1993) and El-Faham (Gamila) (1993) who

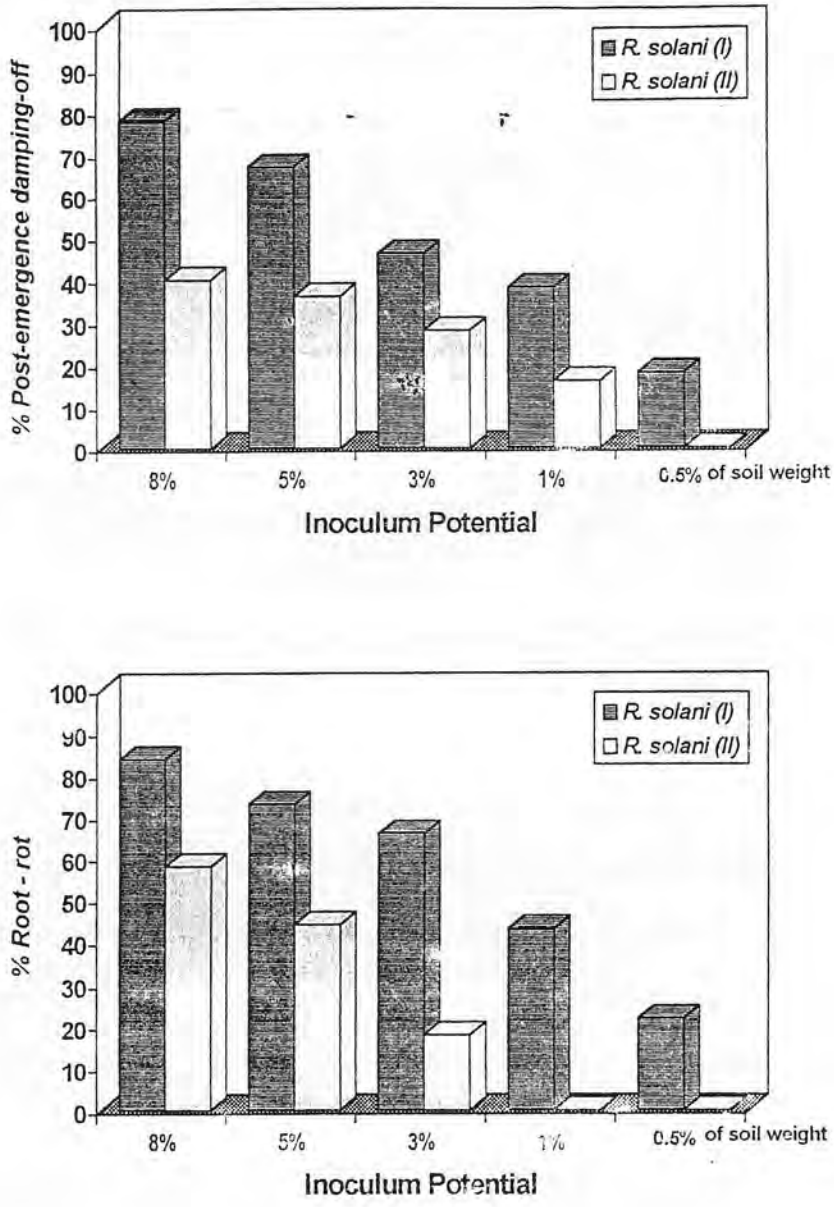

Fig. (1): Pathogenicity and inoculum potential of *R. solani* isolates on broad bean plants.

found that application of *Rh. leguminosarum*, *Rh. meliloti* and *Bradyrhizobium japonicum* as seed dressing or as soil drench reduce the infection with *M. phaseolina*, *R. solani* and *Fusarium spp.* in both leguminous (soybean, mungbean and lentil) and non- leguminous (sunflower) plants.

Table (1): Effect of rhizobial, viral inoculation and fungal infestation on disease severity and roots nodulation.

Treatments	% Post-emergence damping-off	% Root-rot	% Viral infected plants	Nodules weight (g/plant) Fresh	Nodules weight (g/plant) Dry
A- Sterilized Soil:					
Control (1)	0.00	---	---	---	---
R. solani (R)	46.67	68.6	---	---	---
Bean common mosaic virus (BCMV)	0.00	---	71.92	---	---
Rh. leguminosarum (R. leg.)	0.00	---	---	3.96	1.80
R. solani + BCMV	20.00	22.5	53.66	---	---
R. solani + Rh. leg.	13.33	10.0	---	2.72	1.28
BCMV + Rh. leg.	0.00	---	46.63	2.28	1.12
R + BCMV + Rh. leg.	6.67	7.5	50.0	2.35	1.16
B- Non-Sterilized Soil :					
Control (2)	26.33	43.6	---	2.76	1.01
BCMV	13.33	15.6	63.0	2.85	1.13
Rh. leguminosarum	9.99	10.0	---	3.60	1.76
BCMV + Rh. leg.	0.00	4.9	40.0	2.06	0.84
L.S.D. at 0.05	15.10	8.42	13.82	0.23	0.17
L.S.D. at 0.01	20.21	11.35	18.73	0.28	0.20

The lower percentage of fungal infection resulted from viral inoculation may be attributed to an antagonistic effect between fungal and viral infection (Magyarosy and Hancock, 1974; El-Hammady *et al.*, 1983 and Gamal El-Din *et al.*, 1990). Also, Abd El-Mageed (1992) found that, soluble or cell wall bounds protein extracted from hypocotyle, leaves, pods and roots of viral infected bean plants contained more polyglacturinase inhibitor than protein from non-viral infected plants.

Data in Table (1) also emphasized that, post-emergence damping-off or root-rot diseases were significantly decreased by using the combination of BCMV and rhizobial inoculation rather than using each one solely regardless the type of soil.

The viral infection clearly showed mosaic symptoms on broad bean leaves data in Fig. (2) showed the following :

The highest percentage of viral infection occurred in plants inoculated with virus solely in both investigated soils, whereas, the percentage of viral infected plants were decreased in other treatments. The highest significant decrease in viral infected plants was occurred in the treatment inoculated with *Rh. leguminosarum* and BCMV in sterilized and non- sterilized soils. This result is in harmonwith Wahyuni and Randles (1993) and Izaguirre (Mayoral) *et al.,* (1994) who found that the viral infection with bean rugose mosaic virus (BRMV) and cucumber mosaic cucumovirus (CMV) was reduced by prior inoculation of seeds with commercial strains of root nodulating bacteria (*Rhizobium* or *Bradyrhizobium*) and concluded that inoculation by root nodulating bacteria have inhibitive effect on BRMV and CMV.

The percentage of viral infection was decreased in sterilized soil when *R. solani* combined with BCMV, as well as viral infection in the combination of BCMV, *Rhizobium* and *R. solani*. Similar results were obtained by Zink and Duffus (1975), Allam *et al.,* (1978), El-Hammady *et al.,* (1983) and Abd El-Mageed (1995) who reported that several fungi decreased viral infection most probably due to antiviral properties of these fungi.

Data in Table (1) also showed that, the fresh and dry weights of nodules varied according to different treatments. The highest fresh and dry weights of root nodules were recorded in case of rhizobial inoculation solely in both sterilized and non-sterilized soils. While, fresh and dry weights of nodules were decreased when *R. solani* and/or BCMV were combined with *Rh. leguminosarum* and the same trend was observed in non-sterilized soil with viral infection. These results are in harmony with Zahra *et al.,* (1990), Gohar *et al.,* (1991) and Hussein *et al.,* (1993) who reported that rhizobial inoculation of legumes significantly increased numbers and dry weights of nodules/plant.

As regard to fungal infection, Zambolium *et al.,* (1983) and Manninger *et al.,* (1985) reported that the number and weight of *Rhizobium* nodules were greatly reduced by *R. solani* and *F. solani* in soybean. Also, Bhattacharyya and Mukherjee (1990) reported that *Sclerotium rolfsii* reduced the population of *Rhizobium* sp. in the rhizosphere of groundnut, as well as, *S. rolfsii* reduced root nodulation.

Elsheikh and Osman (1995) reported that viral infection with broad bean mottle bromovirus of faba bean significantly decreased number and dry weight of nodules/plant which is the same results observed herein in this investigation.

Effect of rhizobial and/or viral inoculation on microbial counts of broad bean rhizosphere :-

Data in Table (2) indicated that, the populations of soil bacteria and Actinomycetes in all studied treatments were gradually increased with increasing the growth period and reach their maximal values at flowering stage and

Table (2): Periodical changes in bacterial, actinomycetes and fungi counts in non-sterilized soil treatments during different growth stages of broad bean plants.

Treatments	Bacteria ($x10^6$)				Actinomycetes ($x10^6$)				Fungi ($x10^3$)			
	Vegetative stage	Tillering stage	Flowering stage	Maturity stage	Vegetative stage	Tillering stage	Flowering stage	Maturity stage	Vegetative stage	Tillering stage	Flowering stage	Maturity stage
Control (1)	26.4	48.0	68.5	30.75	17.70	27.5	35.9	18.7	92	108	140	84
Bean Common Mosaic Virus (BCMV)	62.6	93.9	122.9	59.4	32.70	46.2	93.5	29.7	44	26	22	18
Rh. leguminosarum (Rh. leg.)	96.0	167.0	219.0	135.0	55.30	99.0	147.0	97.0	74	56	35	22
BCMV + Rh. leg.	82.8	137.5	196.0	132.0	38.15	78.4	132.0	68.9	46	38	26	20

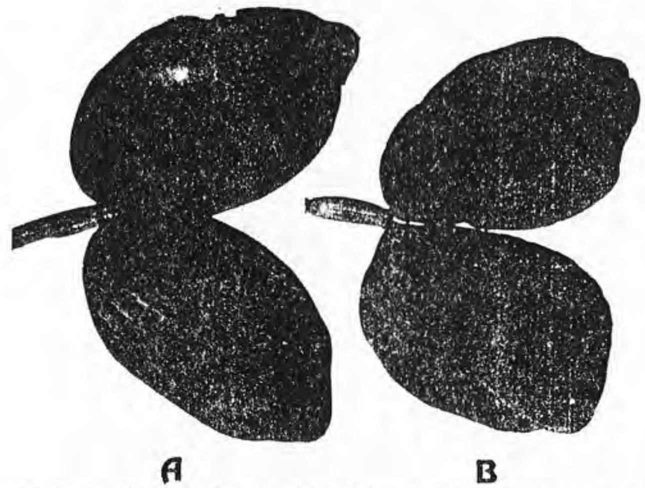

Fig.(2): A- Uninoculated plants (Healthy) B- Mechanical inoculated broad bean plants showed mosaic symptoms.

decreased thereafter. On the contrary, except the control treatment, fungi counts were decreased with increasing the growth period till the end of experiment. Comparing with the control treatment, the bacterial counts were increased in case of rhizobial inoculation either solely or in combination with virus. This trend was observed at all growth stages of broad bean plants. This increase of bacterial counts in case of rhizobial inoculation may be due to nitrogen fixation by *Rh. leguminosarum* which activate the bacterial proliferation in rhizosphere region. These results are in accordance with those obtained by Saelahan *et al.*, (1965), Philips and Torrey (1970) and Huo *et al.*, (1980) who reported that, legume-nodulating bacteria synthesize cytokinene and gibrillins which may be enhance the bacterial proliferation. As regard to viral inoculation effect, Abd El-Mageed (1992) found that, the total bacterial counts in rhizosphere of viral infected plants at flowering stage of bean was higher than that in the rhizosphere of virus-free plants. This may be due to that 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 Stephens, 1989).

Data in Table (2) also showed that, the highest counts of Actinomycetes were recorded in the rhizosphere of inoculated broad bean plants with *Rh. leguminosarum* biovar *viceae* solely and this was true at all growth stages. This increase may be due to the growth promoting substances produced by rhizobial bacteria. Philips and Torrey (1970), Sabelnekova (1979) and Kefford *et al.*, (1980) mentioned that legume-nodulating bacteria synthesize cytokinene and gibrillins which may enhance the growth of various soil micro-organisms and increase the beneficial effect of root exudates in legumes roots. Compared with the control, viral inoculation increased the Actinomycetes counts when inoculated either solely or in combination with *Rh. leguminosarum*. It is importance to notice that the increase of Actinomycetes counts may be reflected on the reduction of post-emergence damping-off and root-rot which previously discussed in Table (1).

Results in Table (2) also showed that, except the control treatment the populations of fungi were decreased with increasing the growth period in all studied treatments.

Bean common mosaic virus when inoculated either solely or in combination with *Rh. leguminosarum* led to decrease the soil fungi populations and this was true at all growth stages compared with rhizobial inoculation solely. This indicates that some root exudates of the viral infected plants may contain some fungi inhibitors (Abd El-Mageed, 1992).

Effect of rhizobial, viral inoculation and fungal infestation on some broad bean growth characters :

Data in Table (3) showed that, plant height was decreased in sterilized soil with either *R. solani* or BCMV solely. Similar result was obtained (in case of viral infection) by Fawzy (1973), Rizkalla (1983) and Fawzy and Abd El-

Mageed (1990). Moreover, Amer *et al.*, (1983) reported that, viral and fungal infection lead to stunting of the vegetative parts of the plant. On the contrary, rhizobial inoculation gave significant increase in plant height when inoculated solely, as well as, when combined with either *R. solani* or bean common mosaic virus.

Data also showed that, in non-sterilized soil treatments, compared with the control, plant height was significantly increased in all studied treatments, while, the highest significant increase was obtained with rhizobial inoculation either solely or in combination with BCMV. This result clearly indicates that *Rh. leguminosarum bv. viceae* can antagonize *R. solani* or BCMV and consequently reduce their harmful effect. Also, Abd El-Latif (Faten) (1994) in her study on the interaction between *R. solani* and *Rh. leguminosarum bv. viceae* obtained similar data.

As regard to leaves number/plant, data recorded in Table (3) emphasized that, the leaves number was decreased in sterilized soil with treatments infested with *R. solani* and/or BCMV. While, number of leaves/plant significantly increased when *Rh. leguminosarum* combined with either *R. solani* or BCMV. On the contrary, number of leaves/plant were significantly increased in inoculated treatment with *Rh. leguminosarum* solely compared with the other investigated treatments. Under non-sterilized soil treatments, a significant increase was observed in the number of leaves/plant specially with rhizobial inoculation either solely or combined with BCMV.

As regard to number of flowers/plant data showthat, in sterilized soil the number of flowers was decreased in the treatment infested with *R. solani* as compared with control (1), while, BCMV infection showed non-significant increase in number of flowers. On the contrary, in other treatments number of flowers was significantly increased and this results appeared clearly with all treatments which contained the rhizobial inoculation except BCMV treatment as well as the treatment inoculated by the combination of *R. solani* + BCMV + *Rh. leguminosarum* as they showed non-significant increase in the number of flowers/plant. This result is in accordance with Amer *et al.*, (1983) and Fawzy and Abd El-Mageed (1990). Also, the number of flowers/plant was the highest in case of rhizobial inoculation solely in non-sterilized soil as compared with other treatments. Compared with the control (2), data showed non-significant increase in number of flowers with BCMV and BCMV + *Rh. leguminosarum* treatments. In this respect, viral infection had 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 (Nour and Nour, 1962).

As regard to rhizobial inoculation, similar results were obtained by Hamdi (1976), Abdel-Nasser *et al.*, (1988) and Gohar *et al.*, (1991) who reported that, rhizobial inoculation of legumes significantly increased the plant height, No. of leaves/plant, no. of flowers and crop yields.

Table (3): Effect of rhizobial, fungal and viral inoculation on some broad bean growth characters.

Treatments	Plant height (cm)	No. of leaves/plant	No. of Flowers/plant	Roots weigh		Shoots weight	
				Fresh	Dry	Fresh	Dry
				g/plant		g/plant	
A- Sterilized Soil:							
Control (1)	69.00	14.00	20.00	1.71	0.84	13.03	2.86
R. solani (R)	66.66	10.40	18.60	1.45	0.46	8.53	2.16
Bean Common Mosaic Virus (BCMV)	67.35	12.00	21.5	1.50	0.72	8.43	2.35
Rh. leguminosarum (Rh. leg.)	101.68	18.96	35.30	3.80	2.01	18.20	4.69
R. solani + BCMV	72.66	12.86	24.68	2.46	0.68	12.86	2.38
R. solani + Rh. leg.	79.35	14.66	28.00	2.68	1.20	13.96	2.22
BCMV + Rh. leg.	77.46	15.35	30.00	2.70	0.80	14.26	2.81
R + BCMV + Rh. leg.	70.00	14.00	22.60	2.70	1.66	17.80	3.94
B- Non-Sterilized Soil :							
Control (2)	60.00	10.65	23.35	1.48	0.64	12.65	2.86
BCMV	71.66	14.00	27.00	1.50	0.70	10.06	2.60
Rh. leguminosarum	87.00	17.35	34.65	4.06	1.83	18.33	4.20
BCMV + Rh. leg.	88.33	16.00	26.00	2.56	1.47	15.26	2.66
L.S.D. at 0.05	8.50	2.67	4.17	0.99	0.351	4.22	0.849
L.S.D. at 0.01	11.37	3.57	5.59	1.32	0.430	5.65	1.13

With respect to fresh and dry weights of roots, in sterilized soil treatments data showed that, *R. solani* or BCMV treatments reduced the fresh and dry weights of roots. These results came in agreement with those of Badr (1978) Amer *et al.*, (1983) and Fawzy and Abd El-Mageed (1990). While, rhizobial inoculation either solely or in combination with BCMV or *R. solani* gave significant increase as compared with the control. The same trend of results was observed in all treatments carried out in non-sterilized soil.

As regard to fresh and dry weights of shoots data clearly indicated that, rhizobial inoculation solely gave the highest fresh and dry weights of shoots compared with other treatments while treatment infested with *R. solani* and/or BCMV gave lower fresh and dry weights of shoots as compared with the control (1). This may be attributed to the effect of viral and/or fungal infection on different physiological processes in plant which lead to stunting of the vegetative parts of the plant. Amer *et al.*, (1983) reported that, single and double infection with BCMV and *Myrothecium verrucaria* markedly decreased the dry weight of bean leaves. A non-significant increase in fresh and dry weights of shoots was recorded in the treatments inoculated by *Rh. leguminosarum* combined with *R. solani* or BCMV while, their combination gave significant increase. The same trend of results was obtained in non-sterilized soil treatments. These results are in harmony with many investigators (Kremer and Patterson, 1983 and Gohar *et al.*, 1991) who reported that, in case of rhizobial inoculation of leguminous plants; the dry weight of plant organs and seed yield were significantly increased. El-Faham (Gamila) (1993) found that application of *Rh. leguminosarum*, *Rh. mliloti* and *Bradyrhizobium japonicum* as seed-dressing with infestation by *R. solani*, *Macrophomina phaseolina* or *Fusarium spp.* improved plant growth characters and gave increase in shoot length and dry weights of plant organs as compared with untreated control. As regard to rhizobium and virus interaction, El-Sheikh and Osman (1995) found that, viral infection with broad bean mottle bromovirus (BBMV) or bean yellow mosaic potyvirus (BYBV) significantly decreased shoot and root dry weight and number of flowers/plant, while, inoculation of both viruses with *Rh. leguminosarum* significantly increased all these parameters.

Effect of rhizobial, viral inoculation and fungal infestation on some chemical constituents of broad bean plants :

Data in Table (4) clearly indicated that, total nitrogen and crude protein were increased in all studied treatments as compared with the control (1) and (2), specially in case of rhizobial inoculation either solely or combined with other treatments and this was obvious under non-sterilized soil. While, when plants inoculated by BCMV, the total nitrogen and crude protein showed moderate increase and this trend was observed in non-sterilized soil. Except the control treatment, fungal infestation solely in sterilized soil showed the lowest values of total nitrogen and crude protein compared with other investigated treatments. These results are in harmony with those reported by Kremer and Patterson (1983), Gohar *et al.*, (1991) and Radhakrishnan and Chatrath (1991) who found that, rhizobial inoculation of legumes crops gave higher increase of nitrogen content and protein than uninoculated ones. As regard to viral infection Fawzy (1973) found that, infection of broad bean plants with PMV and BBMV raised the total nitrogen content of leaves. Amer *et al.*, (1983) also reported that, virus infection (BCMV) increased total nitrogen content in inoculated leaves above healthy ones followed by combined inoculation (BCMV + *Myrothecium verrucaria*). In contrast, they also found that, fungal infestation greatly diminished total nitrogen percentage.

Total phosphorus was increased in all applied treatments in sterilized soil Table (4) as compared to the control specially in case of fungus combined with virus. In non-sterilized soil, rhizobial inoculation combined with viral infection showed the highest value of total phosphorus. Except the control (1), total phosphorus was the lowest value in case of fungal infestation solely in sterilized soil compared with the other treatments which showed moderate increase in total phosphorus content. These results are in agreement with those obtained by El-Shakweer and Barakat (1984) and Hammouda *et al.*, (1991) who found that rhizobial inoculation of legumes crops gave higher increase of phosphorus content than uninoculated ones. Fawzy (1973) found that, infection of broad bean plants with PMV and BBMV increased total phosphorus content in leaves and stems. Whereas, Rizkalla (1983) mentioned that, a reduction in phosphorus ranging from 5.7 to 26.1% and from 1.7 to 27% was observed in broad bean plants infected by BBMV or BYMV, respectively. Also, Fawzy and Abd El-Mageed (1990) studied the effect of infection with combination mixture containing fungi (*F. moniliforme* and *T. roseum*) and viruses (BCMV and BYMV) on P-content of bean plants. They found that, all the studied treatments decreased the percentage of phosphorus with the exception of BCMV + BYMV + *F. moniliforme* treatment, which showed an increase in P-content.

Data in Table (4) also showed that, in sterilized soil treatments chlorophyll a was greatly increased in case of rhizobial inoculation solely as well as when combined with either *R. solani* or BCMV. Whereas, the lowest level of chlorophyll a was observed in case of *R. solani* infection solely. In contrast, viral inoculation either solely or combined with *R. solani* showed moderate decrease in chlorophyll a level, compared with the control.

Concerning with chlorophyll a level in non-sterilized soil treatments results emphasized that rhizobial inoculation either solely or in combination with BCMV gave higher value of chlorophyll a than other treatments, while viral inoculation solely gave the lowest chlorophyll a content.

Data in Table (4) also showed that, chlorophyll b level was increased in case of rhizobial inoculation either solely or in combination with either *R. solani* or BCMV. On the contrary, viral inoculation either solely or combined with *R. solani* decreased chlorophyll b level, as compared with control treatment while, fungal infestation solely slightly increased its content.

Table (4): Effect of rhizobial, fungal and viral inoculation on some chemical constituents in broad bean plants.

Treatments	Total nitrogen %	Crude protein content (%)	Total Phosphorus %	Chlorophyll A	Chlorophyll B	Chlorophyll C	Total carbohydrate mg/g dry matter
				mg/g fresh matter			
A- Sterilized Soil:							
Control (1)	1.95	12.18	0.250	0.347	0.186	0.149	22.00
R. solani (R)	2.42	15.12	0.312	0.289	0.190	0.254	16.50
Bean Common Mosaic Virus (BCMV)	3.50	21.87	0.375	0.335	0.115	0.206	18.28
Rh. leguminosarum (Rh. leg.)	4.75	29.68	0.717	0.618	0.372	0.325	62.00
R. solani + BCMV	4.25	26.56	0.812	0.321	0.118	0.198	20.00
R. solani + Rh. leg.	3.41	21.31	0.562	0.600	0.355	0.299	48.00
BCMV + Rh. leg.	3.87	24.18	0.500	0.603	0.323	0.248	46.00
R + BCMV + Rh. leg.	3.62	22.62	0.395	0.624	0.300	0.260	36.50
B- Non-Sterilized Soil :							
Control (2)	2.25	14.06	0.475	0.395	0.157	0.124	28.00
BCMV	3.47	21.68	0.702	0.347	0.106	0.166	21.00
Rh. leguminosarum	4.87	30.43	0.750	0.656	0.276	0.370	75.00
BCMV + Rh. leg.	4.25	26.56	0.890	0.533	0.213	0.337	60.00

Regarding to chlorophyll b level in non-sterilized soil treatments data showed that, compared with the control (2), rhizobial inoculation solely or combined with BCMV increased chlorophyll b level. In contrast, viral inoculation solely sthe lowest chlorophyll b content.

Regarding to chlorophyll c data showed that chlorophyll c was increased in all studied treatments specially in case of rhizobial inoculation solely as well as when combined with either *R. solani* or BCMV regardless the type of soil, while, chlorophyll c moderately increased in case of *R. solani* or BCMV and both of them in sterilized soil compared with the control and the same result was obtained with respect to viral inoculation solely in non-sterilized soil.

From the previous data regarding to chlorophyll a, b and c it could be concluded that, the three pigments were almostly increased in case of rhizobial inoculation solely, as well as, when *Rhizobium* combined with either *R. solani* or BCMV regardless the type of soil. This result clearly indicates that, *Rh. leguminosarum* biovar *viceae* can antagonize *R. solani* or BCMV and reduce the harmful effect of each pathogen solely.

These results are in accordance with Mahdy (1981) who found that, all determined pigments recorded low values due to soil infestation with *F. oxysporum* f.sp *vasinfectum* in some cotton varieties. While, Rizkalla (1983) recorded that, broad bean wilt and bean yellow mosaic virus reduced the three pigments in infected Giza 1 and 2 samples collected at different intervals of infection. Also, Gamal El-Din *et al.*, (1990) reported that, there was considerable decrease in chlorophylls and carotenoids due to viral inoculation, as well as, infestation with *F. moniliforme* or *T. roseum* or their combination.

Data in Table (4) also showed that, the total carbohydrates was the highest level in case of rhizobial inoculation solely in both investigated soils. While, the lowest level of carbohydrates was observed in case of *R. solani* solely in sterilized soil and with viral inoculation solely in non-sterilized soil. Also, the total carbohydrates was lower than that of control in case of viral inoculation either solely or in combination with *R. solani* in sterilized soil.

On the other hand, rhizobial inoculation combined with either *R. solani* or BCMV showed highly increase in carbohydrate level compared with treatments of each one solely regardless the type of soil. Generally, total carbohydrates was almost proportionated with chlorophylls level in various treatments since the total carbohydrates level was increased with increasing chlorophylls content. Similar results were obtained by Abd El-Mageed (1981 and 1986) who found that, total carbohydrates percentage of the leaves was decreased in case of the infection with *F. moniliforme* in bean plants. Also, Amer *et al.*, (1983) reported that, single infection with BCMV or double infection of bean plants with virus and *Myrothecium verrucaria* decreased the carbohydrates content. Also, Gamal El-Din *et al.*, (1990) found that, when soil was infested with *F. moniliforme* and *T. roseum* in combination the carbohydrates of leaves was decreased.

CONCLUSION

From the results previously discussed, it could be concluded that, rhizobial inoculation should be applied at planting time to decrease the infection with post-emergence damping-off and root-rot diseases in broad bean and to minimize the harmefull effect of viral (BCMV) diseases.

Bean common mosaic virus or *R. solani* solely were found to decrease broad bean growth characters as well as photosynthetic pigments and carbohydrates content. While, their combination decreased these harmfull effects caused by each one solely.

Rhizobial inoculation improved plant growth characters and increased both protein and phosphorus content. Nevertheless, root nodulating bacteria increased photosynthetic pigments and carbohydrates assimilation.

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تأثير تفاعل التلقيح بالرايزوبيوم على الإصابة الفيروسية والفطرية في الفول البلدي

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قسم النبات الزراعي – كلية الزراعة بمشتهر – جامعة الزقازيق/فرع بنها – مصر

في هذا البحث تم دراسة تأثير تفاعل تلقيح بذور الفول البلدى بسلالة الرايزوبيوم *Rh. leguminosarum* biovar *viceae* على أمراض سقوط البادرات وعفن الجذور المتسبب عن فطر الـ *Rhizoctonia solany* والعدوى بفيروس موزايك الفاصوليا العادى Bean common mosaic virus (BCMV) وذلك فى التربة المعقمة وغير المعقمه.

وقد أوضحت النتائج أن العدوى بفطر *R. solani* منفردا أدى إلى حدوث أعلى نسبة إصابة بمرض سقوط البادرات فوق سطح التربة وعفن الجذور ، بينما قلت هذه النسبة عند التلقيح بالرايزوبيوم وفيروس BCMV كل على حده ، وكانت أقل نسبة لكلا المرضين عند التلقيح المزدوج بكل من الرايزوبيوم والفيروس.

أعلى نسبة للإصابة الفيروسية لوحظت عند العدوى بالفيروس منفردا بينما نقصت نسبة الإصابة الفيروسية مع المعاملات الأخرى وخصوصا عند التلقيح بالرايزوبيوم في التربة المعقمة والغير معقمة.

أدى التلقيح بالرايزوبيوم منفردا إلى زيادة الوزن الطازج والجاف للعقد الجذرية ، بينما كان هناك نقص معنوي عند العدوى بفطر *R. solani* أو فيروس BCMV أو كلاهما معا.

أوضحت النتائج زيادة في العدد الكلي للميكروبات والأكتينومايسيتات في حالة التلقيح بالرايزوبيوم سواء منفردا أو مع الفيروس خلال مراحل النمو المختلفة. بينما أدى التلقيح بالفيروس أو الرايزوبيوم أو كلاهما معا إلى نقص في أعداد الفطريات في منطقة الريزوسفير مقارنة بالنباتات الغير ملقحة.

زاد طول النبات ، عدد الأوراق ، عدد الأزهار والوزن الطازج والجاف للمجموع الجذري والخضري نتيجة التلقيح بالرايزوبيوم منفردا أو مع الفطر و الفيروس في كلا نوعي التربة. حدث نقص معنوي لكل الصفات السابقة عند العدوى بالفطر أو الفيروس ، بينما عند العدوى بالفيروس والفطر معا قل هذا النقص مقارنة بكل منهما منفردا.

ولقد أوضحت النتائج زيادة في محتوى المجموع الخضري من النيتروجين والبروتين مع التلقيح بالرايزوبيوم سواء منفردا أو مع الفطر أو الفيروس كل على حده، باستثناء المقارنة ، وجد أن أقل مستوى للنيتروجين والبروتين كان في حالة العدوى بالفطر منفردا في التربة المعقمة.

أعلى نسبة للفوسفور لوحظت عند التلقيح بالفطر والفيروس في التربة المعقمة، والفيروس والرايزوبيوم في التربة الغير معقمة ، بينما أقل نسبة للفوسفور كانت في حالة العدوى الفطر منفردا في التربة المعقمة.

كذلك أدى التلقيح بالرايزوبيوم سواء منفردا أو مع الفطر أو الفيروس إلى زيادة مستوى الكلورفيلات في النبات بغض النظر عن نوع التربة. بينما أقل نسبة لكلورفيل أ لوحظت عند العدوى بالفطر فقط أما أقل نسبة لكلورفيل ب فلوحظت عند العدوى بالفيروس فقط ، وقد أدت العدوى بالفطر والفيروس معا إلى إنخفاض صبغة الكاروتين في النباتات المصابة.

أوضحت النتائج أيضا أن أعلى نسبة للكربوهيدرات كانت مع التلقيح بالرايزوبيوم منفردا في كلا نوعي التربة ، بينما أقل نسبة للكربوهيدرات لوحظت مع العدوى بالفطر منفردا في التربة المعقمة والفيروس منفردا في التربة الغير معقمة.

ومن ناحية أخرى فقد وجد أن التلقيح بالرايزوبيوم في وجود الفطر أو الفيروس أدى إلى زيادة في محتوى الكربوهيدرات بالمقارنة بالعدوى الفطر أو الفيروس منفردين وذلك في التربة المعقمة.