J. Agric. Sci. Mansoura Univ., 28 (3): 2199 - 2212, 2003

TOWARDS INTEGRATED BIOFERTILIZERS MANAGEMENT WITH *RHIZOBIUM*, *AZOTOBACTER* AND MYCORRHIZA FOR ENHANCING THE GROWTH OF FABA BEAN INFECTED WITH BROAD STAIN VIRUS (BBSV)

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ABSTRACT

Pot experiments were conducted to study the effect of individual or mixed inoculation with *R. leguminosarum* bv. *Viceae, Azotobacter choroccum* and phosphate dissolving mycorrhiza (VAM) used to inoculate faba bean infected or non – infected with board bean stain virus (BBSV).

Application of *Rhizobium* alone or in combination with either *Azotobacter* or mycorrhiza led to a significant increase in number and dry weight of nodules, and dry weight of shoots and its total nitrogen content on both healthy or viral infected plants in comparison with their corresponding control. The increase of nitrogen, phosphorus, Photosynthate pigments were also significantly increased after 60 days of planting. In these respect the triple inoculation showed superiority than single or dual inoculation. Separate virus infection, significantly reduced all these parameters. On the other hand a clear recovery was obtained in all nitrogen fixation parameters, as well as in grain yield and its total N contents of disease plant due to application of mixture of biofertilizers .

The seed yield and its N concentration (N%) of diseased plants was significantly enhanced by triple inoculation . The percentage of improvement reached to 137. 27 % and 35.7 % respectively .

INTRODUCTION

Faba bean (*vicia faba. L.*) is one of the most important legume grain legumes in Mediterrean agricultural areas (Buttery *et al.*, 1992). In Egypt, it consumed in large quantities as human food or animal feed, since its dry seeds contain about 30% protein and 65% carbohydrates as main components (Omar *et. al.*, 1990). However, faba beans in most cultivated areas of Egypt are subjected to serious problems caused by virus diseases. Broad bean stain virus is one of the nine widely prevalent viruses recently detected in broad beans in North Africa in which seeds show staining reminiscent (Makouk *et al.*, 1988; and Omar , 1990).

These pathogens adversely affected the symbiotic N2-Fixation as well. Increased environmental awareness has promoted the development of biologiacal means as alternative to chemical used in agricultural process (Sutz *et al.*, 2000). It relies on the potency of beneficial microorganisms (PGPR) to improve plant growth, strengthen biological N– Fixation to colonize the roots and displace the pathogenic microorganisms. One of which is *Azotobacter,* the free- living N2-fixing organs, which live in close association with plants in rhizoshere. Its beneficial effects is attributed mainly to

improvement in root development, an increase of water and mineral uptake by roots, displacment of pathogenic bacteria (Brown, 1974; Okan and Itzigsohn, 1995) . Also, Mycorrhizal fungi (VAM) are known to increase nutrient uptake particularly phosphorus, the essential element for N2- Fixation (El Didamony and ; Abdel – Fattah, 1998 and Subba Roa, *et al.*, 1986).

Ecological interaction between rhizobia and plants growth promoting rhizobacteria (PGPR) have of interest in recent years because of their agronomical implications. Many papers reported, the positive effect of combined inoculation with *Rhizobium* plus *Azotobacter* and / or VAM because its favourable influence on nodule weight and number, plant dry matter accumulation, grain yield and N. content (Daft and Giahmi, 1974; Burns *et. al* 1981; Sairig *et al.*, 1986; Subba Roa *et al.*, 1986; Yahalom *et al.*, 1987; Rodeas *et. al* 1996 and 1999).

Consequently the main purpose of this work was to study under controlled conditions the effect of inoculation with *Azotobacter* and / or VAM on *Rhizobial* symbiosis and growth of healthy and viral infected faba bean plants aimed at clarifying to what extend, these type of co-inoculation treatments can offer a level of protection against viral infection and can that help the plant to overcome the stressful conditions due to disease severity.

MATERIALS AND METHODS

A)Green-house pot experiment.

Soil used in this study was a subsurface layer10-30cm of a clay loam (organic carbon, 0.75%; total nitrogen, 0.11%; PH 8.1; E.C.2.1 dsm $^{-1}$; WHC, 52.2%) belonging to order Entisol (Soil survey staff 1975) and collected from Sakha Agriculture Research Station Farm at Kafr El-Sheikh governorate, Egypt. The soil initially contained 500 VAM spores Kg^{-1} of dry soil. The soil was crushed to pass through a 2-mm sieve. Earthen pots (35 × 40cm) were filled with 10kg clay loam soil. All pots were supplemented with the following chemical fertilizers; Calcium superphosphate (15.5% P2O5) at the rte of 50 kg fed^{-1} before sowing and urea (46.0%N) at the rate of 20 kg fed^{-1} added after being thinned and before irrigation.

Six seeds of faba bean (*Vicia faba* L.var. Giza3) were planted pot^{-1}, and after 8 days, seedlings were thinned to 3 plants pot^{-1}. *Rhizobium leguminosarum* biovar *viceae* strain No. 40 and *Azotobacter Chrococum* were obtained from the stock culture collection of Dept. of Soil Microbiology Sakha Agric. Res. Station. *Rhizobium* and *Azotobacter* strains were cultured on yeast extract mannitol agar (Vincent 1970, Abd El-Malak and Ishac 1968). Inoculation with *Rhizobium* and *Azotobacter* were carried out 7 days after germination by pippeting 2 ml of their bacterial suspensions ($0.8x\ 10^8$ cells/ ml) to the soil around the base of each seedling VA – mycorrhiza inoculum was prepared and added as described by Armanios *et. al.* (1996).

B) Isolation and viral infection

Broad bean stain virus (BBSV) isolated from leaf samples of naturally infected broad bean plants showing different patterns of symptoms, was

collected from Sakha Experimental Agric. Station. Infected broad bean leaves were homogenized in 0.2 phosphate buffer (pH6) with sterilized mortar and pestle , the extract was filtrated through two layers of cheese cloth and used for infection. Seedlings of faba bean plants was inoculated mechanically with infected sap according to Omar et al. (1990)

C) Treatments and determination

Sixteen treatments were conducted on faba bean seedlings as the following:

1)Uninoculated (control), 2) inoculated with *Rhizobium* alone, 3) inoculated with *Azotobacter* alone, 4) inoculated with VA-mycorrhiza alone, 5) inoculated with *Rhizobium* and *Azotobacter*, 6) inoculated with *Rhizobium* and VA-mycorrhiza, 7) inoculated with *Azotobacter* and VA-mycorrhiza, 8) inoculated with combination of *Rhizobium* and *Azotobacter* and VA-mycorrhiza. The same eight treatments were repeated in the presence of the pathogen (BBSV). Each treatment was represented by eight replicates. Pots were watered to maintain the soil at approximately 60% water holding capacity.

Plants of 40 and 80 days old were examined for number and dry weight of nodules (mg/ plant), dry weight of shoots (g/plant), N and P content of the shoots, (Jackson, 1967) total carotenoids and chlorophylls (wettstin, 1957). At harvest (130 days of planting) dry weight of seeds (g/plant) and N and P contents were also determined.

D) Statistical analysis.

All the data were subjected to statistical analysis as described by Snedecor and Chochran (1967) and means were tested according to Duncan's Multiple Range Test (1955).

RESULTS AND DISCUSSION

Data in Tables 1,2,3 and 4 show the effect of rhizobial inoculation combined *Azotobacter* and/ or Mycorrhiza and viral infection with broad bean stain virus (BBSV) as well as interactions on nodule formation, plant growth, seed yield and yield components of faba bean.

(1) Effect on nodulation:

Number and dry matter accumulation in nodules of healthy and plant infected plants with (BBSV) were significantly affected by single or mixed inoculation treatments (Table 1 and 2), however, the magnitude of the effect was influenced by the type of inoculants. The improvement of nodulation pattern was further strengthened in dual inoculation with *Rhizobium* and / or *Azotobacter* or VAM in this respect data showed that VAM was more effective than*Azotobacter*.

Table (1) : Means of number and dry weight of nodules and dry weight of shoots as affected by the interaction between (BBSV) virus(A)and used biofertilizers (B) after 40 days from planting of faba bean plants.

Characters \ Treatments	A \ B	Rh.	Azot	Myco.	Rh.+ Azot.	Rh.+ Myco	Azot.+ Myco	Rh+Azot. + Myco	Control	M.
Number of nodules/plant	Without (-)	77.33^{c}	21.33^{j}	25.33^{i}	78.00^{bc}	80.66^{b}	28.66^{h}	89.33^{a}	21.00^{j}	52.71
	With (+)	56.66^{g}	17.33^{k}	20.00^{j}	60.66^{f}	67.33^{e}	22.00^{j}	74.00^{d}	17.00^{k}	41.88
Dry weight of nodules (mg/P)	Without (-)	140.00^{d}	110.0^{i}	118.00^{e}	144.00^{c}	151.0^{b}	121.00^{e}	161.00^{a}	101.00^{g}	130.75
	With (+)	92.00^{h}	57.00^{k}	66.00^{j}	93.00^{h}	111.0^{f}	77.00^{i}	120.00^{e}	52.00^{l}	83.50
Dry weight of shoots (gr/p)	Without (-)	5.62^{d}	4.50^{i}	4.87^{g}	5.80^{c}	6.87^{b}	5.23^{f}	7.93^{a}	3.68^{n}	5.56
	With (+)	4.27^{k}	3.72^{n}	3.87^{m}	4.40^{j}	4.67^{h}	3.98^{l}	5.35^{e}	2.33^{p}	4.07

Without (-) = Viral non infected plants .
With (+) = Viral infected plants .

Table (2) : Means of number and dry weight of nodules , dry weight of shoots , and percentage of nitrogen and phosph. contents in shoots as affected by the interaction between (BBSV) virus (A) and used biofertilizers (B) after 80 days from planting of faba bean plants .

Characters \ Treatments	A \ B	Rh.	Azot	Myco.	Rh.+ Azot.	Rh.+ Myco	Azot.+ Myco	Rh+Azot. + Myco	Control	M.
Number of nodules/plant	Without (-)	96.00^{c}	27.00^{h}	30.33^{g}	97.33^{c}	103.00^{b}	31.33^{g}	124.66^{a}	25.66^{h}	66.92
	With (+)	45.00^{f}	15.33^{j}	16.00^{j}	44.33^{f}	57.66^{e}	19.33^{i}	63.00^{d}	10.00^{k}	34.46
Dry weight of nodules (mg/p)	Without (-)	377.00^{c}	121.00^{e}	131.00^{e}	378.00^{c}	413.00^{b}	173.00^{d}	441.00^{a}	103.00^{f}	267.21
	With (+)	83.00^{h}	54.00^{j}	58.00^{ij}	86.00^{g}	93.00^{fg}	68.00^{hi}	93.00^{fg}	47.00^{j}	72.75
Dry weight of shoots (gr/p)	Without (-)	9.01^{d}	7.05^{g}	7.73^{f}	9.30^{c}	10.73^{b}	8.83^{e}	12.53^{a}	5.93^{j}	8.95
	With (+)	6.27^{i}	5.47^{l}	5.77^{k}	5.70^{k}	6.87^{h}	5.93^{j}	7.10^{g}	2.70^{m}	5.84
N2% in shoots/p	Without (-)	2.55^{c}	2.07^{f}	2.10^{ef}	2.59^{bc}	2.61^{b}	2.20^{d}	2.78^{a}	1.96^{ih}	2.36
	With (+)	2.12^{cf}	1.92^{hi}	1.95^{h}	2.13^{e}	2.12^{d}	2.00^{j}	2.20^{d}	1.90^{i}	2.03
Phosph.% in shoots/p	Without (-)	0.51^{d}	0.48^{g}	0.53^{b}	0.51^{d}	0.52^{c}	0.53^{b}	0.57^{a}	0.48^{g}	0.51
	With (+)	0.46^{i}	0.45^{j}	0.48^{g}	0.46^{i}	0.49^{f}	0.47^{h}	0.50^{e}	0.43^{k}	0.47

Without (-) = Viral non infected plants .
With (+) = Viral infected plants .

Table (3): Means of dry weight of seeds and percentage of nitrogen and phosph . contents in seeds (130 days), total Chlor and Cart. in leafs (60 days)of faba bean plants as affected by the interaction between (BBSV) virus and used biofertilizers

Treatments / Characters	B / A	Rh.	Azot	Myco.	Rh.+ Azot.	Rh.+ Myco	Azot.+ Myco	Rh+Azot. + Myco	Control	M.
Dry weight of seeds (g/plant)	Without (-)	9.33^{d}	7.13^{h}	8.27^{f}	10.11^{c}	11.09^{b}	8.93^{e}	12.13^{a}	5.20^{i}	9.03
	With (+)	6.30^{g}	4.87^{j}	5.80^{k}	6.70^{i}	7.23^{h}	6.10^{jk}	7.83^{g}	3.30^{m}	6.02
N2% in seeds	Without (-)	3.67^{c}	3.42^{e}	3.51^{d}	3.70^{bc}	3.75^{ab}	3.45^{e}	3.^{80a}	2.93^{j}	3.53
	With (+)	3.17^{g}	2.98^{ij}	3.00^{i}	3.04^{i}	3.^{21fg}	2.98^{ij}	3.23^{f}	2.38^{k}	3.00
Phosph. % in seeds	Without (-)	0.56^{e}	0.51^{g}	0.59^{d}	0.56^{e}	0.64^{b}	0.62^{c}	0.70^{a}	0.50^{h}	0.59
	With (+)	0.51^{g}	0.47^{j}	0.53^{f}	0.52^{f}	0.57^{e}	0.52^{f}	0.60^{d}	0.45^{j}	0.52
Total chlorophylls (mg/g fresh weight)	Without (-)	2.14d^{e}	2.10^{e}	2.18^{c}	2.15^{d}	2.21^{b}	2.19^{b}	2.28^{a}	1.98^{i}	2.15
	With (+)	1.80^{h}	1.68^{j}	1.73^{i}	1.71^{ig}	1.86^{g}	1.78^{h}	1.87^{g}	1.56^{k}	1.75
Carotenoides (mg/g fresh weight)	Without (-)	0.37^{e}	0.35^{g}	0.36^{f}	0.38^{d}	0.40^{b}	0.39^{c}	0.43^{a}	0.29^{k}	0.37
	With (+)	0.30^{j}	0.26^{l}	0.29^{k}	0.30^{j}	0.31^{i}	0.31^{i}	0.34^{h}	0.20^{m}	0.29

Table (4) : Means of number and dry weight of nodules, dry weight of shoots , percentage of N2 and phosph . in shoots as affected by (BBSV) virus and different combination of biofertilizers after 40 and 80 days from planting of faba bean plants .

Treatments	Sampling date (days)	Virus infection (A)			Biofertilizers (B)									A+B interaction
Characters		Without	With	Sig.	Rh.	Azot.	Myco.	Rh+ Azot	Rh+Myco	Azot+ Myco.	Rh+Azot. + Myco.	Control	Sig	Sig
Number of nodules/p	40	52.71	41.886	**	67.00^{d}	19.33^{g}	22.67^{f}	69.33^{c}	74.00^{b}	25.33^{e}	81.67^{a}	19.08^{g}	**	**
	80	66.92	34.460	**	70.50^{d}	21.17^{g}	23.17^{f}	73.33^{c}	80.33^{b}	25.33^{e}	93.83^{a}	17.33^{h}	**	**
Dry weight of nodules mg/p	40	130.75	83.500	**	116.00^{c}	83.50^{f}	92.00^{e}	118.50^{c}	131.00^{b}	99.00^{d}	140.50^{a}	76.50^{g}	**	**
	80	276.21	72.750	**	230.00^{c}	87.50^{f}	94.50^{e}	232.00^{c}	253.00^{b}	120.50^{d}	267.00^{a}	75.00^{g}	**	**
Dry weight of shoots gr/p	40	5.56	4.07	**	4.94^{d}	4.11^{g}	4.37^{f}	5.10^{c}	5.77^{b}	4.61^{e}	6.64^{a}	3.01^{h}	**	**
	80	8.95	5.84	**	7.63^{d}	6.48^{f}	6.75^{e}	7.97^{c}	8.80^{b}	7.38^{d}	9.82^{a}	4.31^{g}	**	**
N2 % in shoots / p	40	2.45	2.14	**	2.41^{bc}	2.46^{f}	2.11^{e}	2.45^{b}	2.40^{c}	2.19^{g}	2.64^{a}	1.97^{g}	**	N.S.
	80		2.06	**	2.34^{c}	1.99^{f}	2.03^{e}	2.36^{c}	2.41^{b}	2.10^{d}	2.50^{a}	1.93^{g}	**	**
Phosph. % in shoots / p	40	0.50	0.47	**	0.48^{c}	0.46^{d}	0.49^{bc}	0.48^{c}	0.50^{ab}	0.50^{ab}	0.51^{a}	0.45^{d}	**	N.S.
	80	0.51	0.47	**	0.49^{d}	0.46^{f}	0.51^{b}	0.48^{e}	0.50^{c}	0.50^{c}	0.53^{a}	0.45^{g}	**	**

Means designated by the same letter are not significantly different at 0.05 levels according to Duncan's Multiple Range Test (1955) .

Sig. = Significance. ** and N.S. indicates P < 0.01 and not significant , respectively .

These was true at the two investigation periods (40 and 80) days after planting time (DAP). Application of triple inoculum that contain *Rhizobium*, *Azotobacter* and VAM caused a further average increase nodule formation on both healthy and diseased plants, which showed higher significance than those of their corresponding counterparts that received single or dual inoculation. This results were confirmed by Burns *et al.* 1981; Armanios *et al.* 1996 and Rodelas *et al.* 1999.

The obtained data revealed that when healthy and (BBSV) infected faba beans were inoculated with any biofertilizer especially that contain *Rhizobium*, healthy faba bean produced considerable greater numbers of nodules than did (BBSV) diseased faba bean. These susceptibility to rhizobial inoculation decreased as the plant became older. For example when healthy and viral infected plants of faba bean inoculated with *Rhizobium*, they produced 77.33 and 56.66 nods/plant respectively after 40(DAP) time. These figures represents a decrease of 36.5% in susceptibility to rhizobial inoculation (in nodule formation) on (BBSV) diseased plants, at early stages of growth with loss in nodular weight of about 52.1%. Such susceptibility to *Rhizobium* inoculation decreased to 133.3% at later stages of growth accompanied with loss in weight of nodules reached to 354.2%. The same trend was observed for all inoculation treatments. The average loss in nodule formation due to viral infection was 25.6% and 94.1% after 40 and 80 days (DAP), respectively. The corresponding values for noduler tissues were 56.6% and 267.3% respectively.

Results obtained herein, revealed inconsistency in the nodulation pattern for inoculation with *Azotobacter* or VAM and their mixture in the absence of *Rhizobium,* being very less improvement in nodule formation but achieved large amount of bacteroidal tissues in their nodules with average weight of 5.58 mg/nod and 3.25 mg/nod for healthy and diseased plants respectively after 40 (DAP) time in dual inoculation with Azoto. And VAM. The corresponding values for *Rhizobium* were 3.9 and 1.84 mg/ nod, respectively. The larger size of nodular tissue probably due to the better environment conditions in the nodules afforded by *Azotobacter* and VAM, e.g. presence of higher concentration of leghaemoglobin and/or the better supply of nodules with photosynthate by the plant.

(2) Effect on growth parameters, nitrogen and phosphorous content of plant.

All biofertilizers applied to faba bean either in a form of single or mixture exerted clear effects on plant growth and their total N and P content on both healthy or (BBSV) diseased plants (Table 1 and 2). The stimulation, however varied according to the type of inoculants and the age of plants. Triple inoculation still showed highly significant increase in shoot dry matter in healthy and diseased plants followed by dual inoculations of *Rhizobium* and *Azotobacter* and/or VAM., while single inoculations were the least in these respect. A clear recovery in shoot dry weight of (BBSV) in infected plants, inoculated with triple inoculation that can reach to the level of healthy plants which inoculated by *Azotobacter* or VAM and their mixture. The N and P

concentration (%) of shoot fraction did not statistically improved by any type of inoculation treatments (Table 1 and 2) after 40 (DAP) time. By aging of plant to 80 days, these two parameters were significantly enhanced especialiy by co-inoculation with *Rhizobium and Azotobacter* or VAM (Punj and Gupta 1988 and Aranios *et al.*, 1996). It is worthy to mention that total N content of plant was strongly correlated to inoculation with *Rhizobium* (Table 4) beside the vital role of phosphate dissolving VAM, since phosphorous is essential for energy transfer (ATP), which in turn is essential for the operation of the nitrogenase system (Vladimir *et al.*, 2001).

3) Photosynthate pigments (PG)

Either viral infection or *Rhizobium* inoculation combined with *Azotobacter* and/or VAM significantly affected photosynthatic pigment (Total chlorophyll and carotenoid) (Table-3). This study demonstrate a clear recovery in (PG) due to application of different biofertilizers used in the experiment in both healthy and (BBSV) diseased faba bean plants (Khafagy 1991). These could be clarified from the calculated data from which the following could be concluded:

- Reduction of 27% in chlorophyll synthesis due to (BBSV) viral infection in control treatment (1.98 – 1.56)
- Reduction ranged from (18.8 – 26% in chlorophyll synthesis in inoculated treatments (comparison between healthy and diseased plants, both are inoculated with PGPR).
- An increase in chlorophyll synthesis ranged from (6-15.1%) due to inoculation of healthy plants in comparison with their corresponding healthy control (1.98)
- An increase ranged from (7.7–19.9%) in chlorophyll synthesis due to inoculation of diseased plant in comparison with corresponding control (1.58).

The parallel values of carotenoid pigments were (45%), (23.3-3.4.6%), (20.6 – 48.30%) and (30 – 70%) respectively.

4) Grain yield and its N- content

Concerning the grain yield and its total N- Content of healthy and viral infected faba bean, results showed that inoculation with any biofertilizer produced significant increase in comparison with their corresponding control as shown in (Table 5 and 6). In these respect the triple inoculation was found to be highly effective than dual or single inoculation. There are many possibilities to explain this data. These mixed inoculum promoted the growth of faba been, increasing dry matter accumulation in all plant parts, including nodulation.

This faster growth rate resulted in higher of N- plant, while the concentration of this nutrient (%N) in plant tissue remained similar to that found in control plants, suggesting that all additional N- absorbed was used to aid plant growth and development (Armanios *et al.*, 1996).

Table (5) : Means of dry weight of nodules, percentage of nitrogen and phosph . content in seed (130 days), total chlor . and total carotenoides in (60 days) of faba bean plants as affected by (BBSV) virus and different combinations of biofertilizers .

Treatments	Sampling date (days)	Virus infection (A)			Biofertilizers (B)								
Characters		Without	With	Sig.	Rh.	Azot.	Myco.	Rh+ Azot	Rh+Myco	Azot+ Myco.	Rh+Azot.+ Myco.	Control	Sig
Dry weight of seeds (g/plant)	130	9.03	6.02	**	7.82^{d}	6.00^{g}	7.04^{f}	8.41^{c}	9.16^{b}	7.52^{e}	9.98^{a}	4.25^{h}	**
N2 % in seeds	130	3.53	3.00	**	3.42^{b}	3.20^{e}	3.26^{a}	3.37^{c}	3.48^{a}	$3.22d^{e}$	3.52^{a}	2.61^{f}	**
Phosph.% in seeds	130	0.59	0.52	*	0.53^{f}	0.49^{g}	0.56^{d}	0.54^{e}	0.60^{b}	0.57^{c}	0.65^{a}	0.48^{h}	**
Total chlorophylls	60	2.15	1.75	**	1.97^{cd}	1.89^{f}	1.96^{d}	1.93^{e}	2.03^{b}	1.98^{c}	2.07^{a}	1.77^{g}	**
Total carotenoides (mg/g fresh weight)	60	0.37	0.29	**	0.33^{cd}	0.30^{e}	0.32^{d}	0.34^{bc}	0.35^{b}	0.35^{b}	0.38^{a}	0.24^{f}	**

Means designated by the same letter are not significantly different at 0.05 levels according to Duncan's Multiple Range Test (1955) .

Sig. = Significance. and N.S. indicates P < 0.05, and not significant , respectively .**

Table (6) : Percentage of changes in seed yield due to inoculation with different biofertilizers (in the presence or absence of viral infection)

Treatments	BBSV	Rh.	Azot.	Myco.	Rh.+Azot.	Rh.+Myco.	Azot.+Myco.	Rh.+Azot.+ Myco.
Dry weight of seeds (gm/plant)	-	79.40	37.12	59.04	94.42	113.27	71.73	133.27
	+	90.90	47.58	75.75	103.03	119.09	84.85	137.27

Without (-) = Viral non infected plants .
With (+) = Viral infected plants .

$$\text{Without (-)} = \frac{\text{treatment} - \text{control (non-infected with BBSV)}}{\text{control (non - infected with BBSV)}} \times 100$$

$$\text{With (+)} = \frac{\text{treatment} - \text{control (infected with BBSV)}}{\text{control (infected with BBSV)}} \times 100$$

The results presented in this paper are in agreement with previous work which has shown that several plant growth – promoting rhizobacteria exert significant effects on *Rhizobium* legume symbiosis which are only observed when certain combinations of these organisms are used (Burns *et al.*. 1981; Polonenko *et al.*, 1987; Subba-Roa *et al.*, 1986; Chanway *et al.*, 1989 and Vladimir *et al.*, 2000). The positive effect of co-inoculation treatment on healthy and diseased faba bean plants could be related to the role of *Azotobacter* and VAM on symbiotic performance of *R. leguminosarum* bv. *Viceae* and plant growth. The ability of *Azotobacter* to change the root morphology and plant growth rate has been widely described and commonly related to the production of biologically active substance by these bacteria (Becking 1992). The synthesis and exertion of plant growth regulators beside the increase of available phosphorus in plant rhizosphere afforded by VAM explain the earlier nodulation, increase in total nodule number and earlier onset of N_2- fixation that led to the improvement of Rhizobium – legume symbiosis. Also, it has been established that plant growth regulators, such as cytokinins, inhibit the loss of some nutrients, e.g. Cu, B, Zn and No_2- from leaves (Mauk and Nooden 1992). In addition, some strains of *Azotobacter*, strongly enhanced specific uptake of Ca and Mg concentrations in faba bean shoots (Rodelas *et al.*, 1999). All these minerals are necessary to biological N_2- fixation process. Again the combined symbiosis of *Rhizobium* and VAM have a synergistic effect, as the plant growing better with both than sum of the separate effects (Table 4 and 5), because the nitrogen fixation has high phosphorus demand, so the two are closely linked (El. Didamony and Abd El Fattah 1998)

In contrast separate viral infection reduced the leaf area, stem length and the highest reduction was found in root length (7.5%) the location of nodule formation was reported by (Sidaros *et. al.* 1991). In addition all the N_2-fixation parameters are inhibited due to viral infection (Table 3 and 4), (Tu *et. al.* 1970) found that the excess of nitrogen compounds (free amino acid) in nodules of SMV – infected soybean alter C/N ratio and inhibit the normal rate of N-Fixation.

On the other hand a clear recovery was obtained in all N_2- fixation parameters, photosynthate pigment and total P content, as well as grain yield and its total N content of diseased plants due to inoculation treatments. These could be clarified from the obtained data presented in (Tables 2 and 3). Taking in our consideration the grain yield and its N-content as a net result of many interacting factors such as Biological N_2-fixation, plant growth regulators phosphate solubilizers, pathogenicity due to viral infection beside environmental factors and legume crop. The diseased plants produced only 3.3 gm/ plant with total N2% of 2.33. These values raised to 7.83gm/plant with total N_2 % 3.23, respectively due triple inoculation treatment. These figures represent an increase of 137.27% and 35.7% , respectively.

In brief we consider that information obtained from the experiments indicated that nodulating and fixing ability of the rhizobia is influenced by the legume host, mycorrhiza and *Azotobacter* (PGPR). These quadrangular interaction is of particular interest for future research and will be very helpful

for design of successful mixed inoculant that can promote plant growth beside their role in providing nature, safe and effective of durable type of protection against viral diseases.

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الاتجاه نحو استخدام المخصبات الحيوية الريزوبيا والازوتوباكتر والميكورهيزا لتحسين نمو الفول البلدى المصاب بفيروس تلون البذور

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*** المعمل البكتيرى بمحطة البحوث الزراعية بسخا معهد بحوث الأراضي والمياه والبيئة – الجيزة – مصر**

**** قسم النيات الزراعى – كلية الزراعة بشبين الكوم – جامعة المنوفية**

أقيمت تجربة أصص في الصوبة السلكية بمحطة البحوث الزراعية بسخا لدراسة أثر التلقيح البكتيرى لريزوبيا الفول البلدى والازوتوباكتر والميكورهيزا كمخصبات حيوية بصورة منفردة أو ثنائية أو ثلاثية (خليط) وذلك بتلقيح الفول البلدى (جيزة ٣) في وجود وعدم وجود الاصابة بفيروس تلون البذور.

وقد أظهرت النتائج أن التلقيح بريزوبيا الفول البلدى بمفردها أو مع كل من الازوتوباكتر والميكورهيزا أو الخليط أدى الى زيادة معنوية في عدد ووزن العقد الجذرية والوزن الجاف للمسيقان والمحتوى الكلي للنيتروجين في النباتات السليمة وكذا المصابة بالفيروس مقارنة بالكنترول المقارن له (بعد ٤٠ و ٨٠ يوم من الزراعة)، وقد تفوق التلقيح بخليط المخصبات الحيوية الثلاثة (الريزوبيا + الازوتوباكتر + الميكورهيزا) عن اللقاحات الفردية أو الثنائية وظهر ذلك في زيادة النيتروجين والفوسفات والصبغات النباتية (الكاروتينات والكلوروفيل) بعد ٦٠ يوم من الزراعة.

وفي الناحية الأخرى حدث تحسن واضح في قياسات وزن البذور (جرام/نبات) والمحتوى الكلي للنيتروجين في النباتات المصابة بالفيروس وذلك للدور السهام الذي تقوم به كلا من الازوتوباكتر والميكورهيزا لتنشيطها نمو النباتات وزيادة كفاءة عمليات التثبيت الحيوى للأزوت الجوى بواسطة الريزوبيا. وكانت النسبة المئوية للتحسن في وزن البذور حققت ١٣٧,٢٧% وفي المحتوى الكلي للنيتروجين ٣٥,٧% للنباتات المصابة بالفيروس والملقحة بخليط المخصبات الحيوية الثلاثة مقارنة بالنباتات المصابة الفيروس والغير ملقحة بتلك المخصبات الحيوية الثلاثة.