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The Effectiveness of The Bacteria *Rhizobium Leguminosarum* Against Bean Yellow Mosaic (BYMV) Potyvirus Infecting Faba Bean (*Vicia faba* L.) Plants.

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Abstract

A virus causing different symptoms of mosaic, mottling, leaf rolling and distortion in (*Vicia faba*) was found in various fields in Menofia Governorate, Egypt. The virus isolate was detected and identified to bean yellow mosaic virus (BYMV) using symptomatology, indicator plants, serological tests (DAS-ELISA and Dot blot immunoassay (DBIA)) and Light microscope. Also, the study conduct to evaluate the efficiency of two strain of *Rhizobium leguminosarum* (*R. leguminosarum* strain MNF-EM-R4 and *R. leguminosarum* bv.*viciae* strain MNF-EM-R9) for inducing systemic acquired resistance in faba bean to bean yellow mosaic virus (BYMV).Our findings showed that grown faba bean plants from treated seeds with rhizobia and inoculated with BYMV presented a imperative reduction in disease occurrence percentage and ELISA reads. While, these treatments caused an increase in Peroxidase enzyme activity compared with control. SDS-PAGE analysis showed that treatment with *R. leguminosarum* strain MNF-EM-R4 showed one band, this band did not appear in negative control, positive control and treatment with *R. leguminosarum* bv. *viciae* strain MNF-EM-R9 at molecular weight (40 kDa) .Treatments with *R. leguminosarum* strain MNF-EM-R4 and treatment with *R. leguminosarum* bv. *viciae* strain MNF-EM-R9 showed one band did not appear in negative, positive control, at molecular weight (85 kDa).

Key words: *Vicia faba*, BYMV, ELISA, SAR, *Rhizobium leguminosarum* and SDS-Page

Introduction

Faba bean plant is one of food legume crop in the most countries of the world. Because of it has a lot of protein content, it can be eaten as green or dried seeds as part of a healthy diet (Robinson *et al*., 2019). In Egypt, this crop is recognized to be naturally infected by various viruses. From them, Bean Yellow Mosaic (BYMV) belongs to *Potyvirus* genus in *Potyviridae* family of plant viruses (Zeid, 2016). Symptoms induced by BYMV on Faba bean plants were, mosaic, deformation of the new leaves, leading to stunting of plants, reduction of nodulation and considerable yield losses (Efaisha, 2005 ; Zeid, 2016 ; Moury and Desbiez, 2020 and Hosny, *et al*., 2021).

Control of plant viral diseases can be accomplished over inducing plant defense mechanisms, e.g., systemic acquired resistance (SAR) (Ryals, *et al*., 1994). SAR to viral infection can be recognized by using non-pathogenic rhizobacteria (Van Loon *et al*., 1998). Useful rhizobacteria can stimulate plant growth directly over releasing secondary metabolites that have the ability to facilitate the approval of certain nutrients from the root environment (Plant Growth Promoting Microorganisms, PGPM) and indirectly over SAR in plants against pathogens, including viruses(Whipps, 2004 and Elbadry, *et al*., 2006). Between of PGPM, *Rhizobium* spp. Were reported to activate plant defense compounds(Phytoalexins, phenols, pathogensis-related protein genes, flavonoids and others) related with disease management of alfalfa and beans (Dakora *et al*., 1993 and Dakora, 2003).

One of the enzymes involved in the plant defensive response to pathogen infection is peroxidase, which is generally the first to show changes in activity. Peroxidase activity is stimulated the most in hypersensitive hosts, while improved activity has also been documented in hosts that allow systemic infection (Mojca *et al*., 2001).

The aggregation of pathogenesis-related (PR) proteins, which are normally thought to be indicators of the defensive response, was linked to an increase in resistance (Ward *et al*., 1991).Several proteins are induced in treated plants which are commonly mentioned to as "pathogenesis-related proteins (PRs). These PRs are classified as plant-coded proteins that are induced in diseased or associated states (Van Loon *et al.*, 1994**)** induced locally and systemically in the infected leaves and are linked to the improvement of systemic acquired resistance (SAR) against increased infection by bacteria, fungi, and viruses

. The induction of PR proteins in several plant tissues is one of the main biochemical and molecular actions when plant are subjected to infections with pathogens for example viroid, viruses, fungi and bacteria (Van Loon, 1997). When plants are attacked by different pathogens, one of the major biochemical and molecular responses is the induction of PR proteins in several plant tissues (Van Loon, 1997).

Therefore, this study attempted to valuate the efficiency of a seed treatment with a *R. Leguminosarum* in reduction of BYMV effects on faba bean plants.

2. Material and methods:

2.1. Source of Bean yellow mosaic virus isolate:

BYMV was isolated from *Vicia faba* L. cv.(Balady) plants that were naturally infected and gathered from garden of faculty of agriculture, Menoufia University. Leaf samples showing mosaic, malformation and leaf curling symptoms, were considered to be caused by virus infection, suspected in following investigates.

2.2. Biological identification (Host range and symptomatology):

The leaf samples were homogenised with phosphate buffer (1:5 w/v, 0.1 M, 0.1ML, pH 7.2) in a sterile mortar (Mahdy *et al.,* 2007). The sap was then filtered twice using a double sheet of cheesecloth. The sap used to mechanical inoculation into healthy indicator plants as: *Vicia faba* L.cv Balady, *Phaseolus vulgaris* L.cv karnk, *Pisum sativum* L.cv Mister B, *Chenopodium album*, *Ch. Quinoa*, *Datura stramonium* L. and *Nicotiana glutinosa* L .

2.3. Serological identification of BYMV isolate:

Serological assays were done using direct ELISA, positive reaction obtained with the specific antiserum as described by (Clark and Adams, 1977) and Dot blot immunobinding assay was used as described by (Lin *et al*., 1990).

2.4. Light microscopy:

In the LM laboratory FARP, Faculty of Agriculture Research Park-Cairo University, anatomical changes caused by virus infection were explored using faba bean leaves.

2.5. Seeds sterilization and bactrialization:

Surface sterilization of faba bean seeds was done in stages with 70% (v/v) ethyl alcohol (30 sec.) and 7% (w/v) calcium hypochlorite solution (30 min.) before being rinsed three times. The seeds were kept in a 100mL beaker with 50mL of sterile distilled water overnight (SDW). The water was removed after soaking, and the seeds were cleaned 3-4 times with SDW. The sterilized seeds were submerged in a Rhizobia suspension for two strain of *R. leguminosarum* (*R. leguminosarum* strain MNF-EM-R4 and *R. leguminosarum* bv. *viciae* strain MNF-EM-R9), these isolates identified by(Enas, *et al*., 2020) for one hour then leaved seeds at room temperature to dry in a sterilized petri dish. Five seeds were sown in a sterile mixture of soil and sand (2:1) in plastic pots. Treatments were arranged as follows: (T1) Healthy plants without BYMV infection and bacterial inoculation (negative control), (T2) Infected with BYMV without rhizobia inoculation (positive control), (T3) Inoculated by *Rhizobium leguminosarum* strain MNF-EM-R4 and infected with BYMV and (T4) Inoculated by *Rhizobium leguminosarum* bv.*viciae* strain MNF-EM-R9 and infected with BYMV virus. The plants of T2, T3 and T4 were mechanically inoculated with BYMV after two weeks of germination and grew in a Greenhouse for six weeks at 25 ±2 ºC. Three replicates in each.

Evaluation of *Rhizobium leguminosarum* for induction of systemic acquired resistance to BYMV under greenhouse:

Results of SAR was detected by assessment of Percentage disease incidence, ELISA absorbance readings, number of nodules and biochemical (Antiviral proteins and activity of peroxidase and polyphenol oxidase).

2.6. Disease incidence (DI %) :

45 days after planting, All plants with BYMV symptoms were documented. The following calculation was used to estimate the DI: DI% = (Number of symptomatic plants/Total number of plants) x 100 (Reddy *et al*., 1983).

2.7. Determination of Peroxidase enzyme activity:

Peroxidase activity was determined using the method defined by (Fehrman and Dimond, 1967)**.** Aliquots supernatants were analyzed for measuring the peroxidase activity by SPEKOL spectrophotometer at 470 nm. Three replicates were used in each treatment This study was conducted in Central Lab, Faculty of Agriculture, Menoufia University.

2.8. Protein Electrophoresis:

This trail was done to exhibit the reacted antiviral proteins against BYMV infection which initiated because of treating faba bean leaves with *R. leguminosarum* comparing with those initiated in faba bean leaves of healthy and infected only with BYMV. In this respect, electrophoretic analysis using SDS-Page technique as described by (Laemmli, 1970) was used for this purpose. Screening for induced systemic resistance against BYMV infection by making rabid isolation of initiated antiviral proteins and others according to the described protocol by (Bollag *et al*., 1996) who used SDS-Page analysis.

Statically analysis:

One-way analysis of modification (ANOVA) was used to analyze the data, following by LSD test for mean separation. Statical significance was defined as P value <0.05 (CoStat-statistic software, CoHort software) .

Results

3.1. The isolate of Bean yellow mosaic virus:

BYMV was isolated from naturally infected *Vicia faba* L. cv. (Balady) plants, were collected from

garden of faculty of agriculture, Menoufia University. Leaf samples showing mosaic, malformation , leaf curling symptoms and reduction of root nodulation as clear in (Fig ,1).

Fig (1): **A&B**: Green mosaic, Leaf roll and malformation on infected faba bean leaves. **C**: Infected root of infected faba bean plant, showing reduction of root nodulation. D: Roots of healthy faba bean plant.

3.2. Biological identification:

The virus isolate was mechanically inoculated into seven plant species and cultivars from three different families. Different plants' reactions to BYMV were divided into different categories according to their reaction (Table.1and Fig. 2).

Table (1): Susceptibility to mechanical inoculation with the isolated BYMV and responsiveness of different plants, under greenhouse conditions.

Host plant tested				Symptoms induced	ELISA test
Family	Scientific name	English name	Variety		
Chenopodiaceae	*Chenopodium album*	Ouares	—	CLL	+
	Ch. quinoa.	Gooses Foot	—	CLL	+
Leguminosae	*Phaseolus vulgaris* L.	Common bean	Karnk	SYM &Bl & LD	+
	Pisum sativum L.	Garden pea	Mister B	VC& M	+
	Vicia fabae L.	Broad bean	Balady	GM &LR	+
Solanaceae	*Datura stramonium L.*	Jimson-weed		NLL	+
	Nicotiana glutinosa L.	Wirginia plant		0	-

Abbreviation of symptoms:

CLL = Chlorotic local lesion
LD = Leaf deformation
O = No symptoms
VC = Vein clorosis
GM=Green mosaic
LR = Leaf roll
BL = Blisters
M= Mosaic
SYM= Systemic yellow mosaic
+ = Positive reaction
- = Negative reaction
NLL = Necrotic local lesion

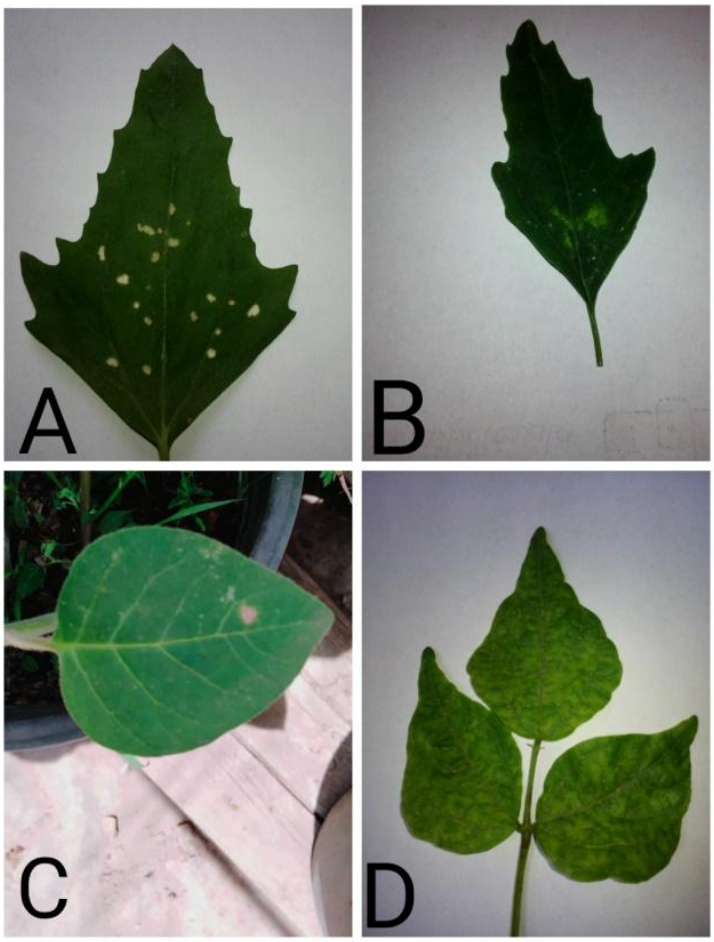

Fig. (2): Response of some indicator hosts to mechanical inoculation with the BYMV isolate. **A**: Chlorotic local lesions on *Ch. quinoa* L. B: Chlorotic local lesions on *Ch.album* L.**C:** Necrotic local lesion on *Datura stramonium* L. **D** : Systemic yellow mosaic, blisters and leaf deformations on *Phaseolus vulgaris* L.

3.3. Serological identification:

The isolated virus was detected by DAS-ELISA, positive reaction obtained with the specific antiserum. BYMV readily detect immunologically using Dot blot technique (DBIA) on nitrocellulose membrane which investigated with polyclonal antisera diluted 1/1000 in TPS buffer and goat anti rabbit alkaline phosphates conjugate diluted 1/8000, was applied as secondary antibody (Fig.3,A).

3.4. Light microscopy:

Semi thin sections of healthy, infected leaves with virus were examined by light microscope after contrast staining. The investigations revealed large difference between healthy and infected tissues. Mesophyll layers (Photosynthetic cells) was reduced and compact in infected plants and the size of plaside cells became short and compact. Also spongy cells were unorganized when compared with healthy ones. The phloem layers and the xylem arms number were reduced and disorganized in the infected cells than in healthy ones (Fig.3 A and B).

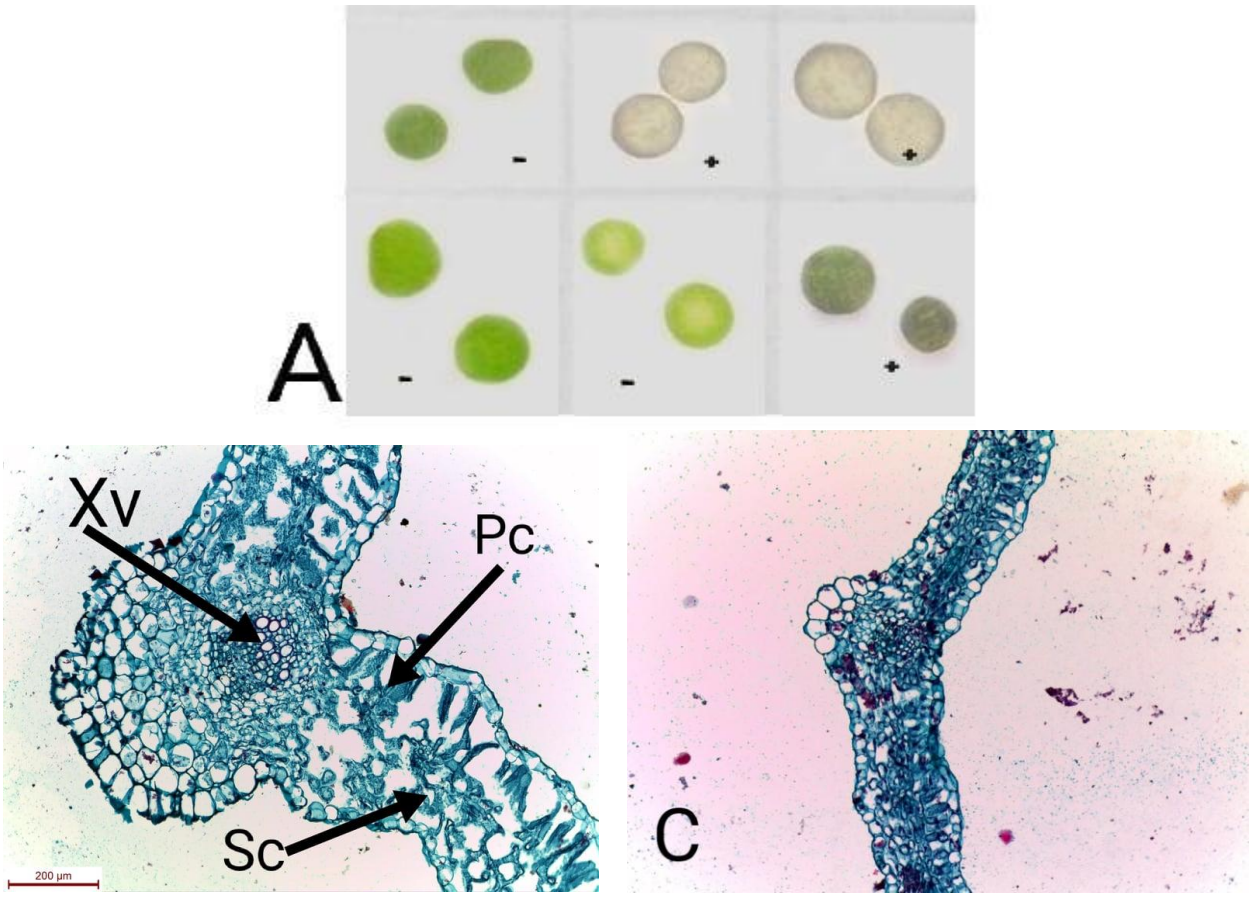

Fig (3). A: Dot Blot Immunoassay for BYMV precipitation against specific IgG- BYMV polyclonal. (+: Positive samples -: Negative samples) **B:** A: Light micrograph of healthy leaves (**Pc**: Plaside cells, **Sc :** Spongy cell and **Xv**: Xylem vessels) **C:** Light micrograph of infected leaves showing reducing and compacting of cells (Pc, Sc) and number of xylem arms and phloem layers

3.5. Disease incidence:

BYMV-inoculated plants grown from rhizobium- treated seeds showed a considerable decreasing (P=0.05) in disease incidence (DI%) when compared to plants emerged from non-rhizobium treated seeds (Table 2). Data present in table (2) indicated that plants emerged from Rhizopium- inoculated seeds (T4) showed considerable decreasing in DI (22.06 %) followed by those grown from rhizobium treated seeds, 17.27% (T3) compared with 91.33% for the challenged control (infected with virus and without Rhizopium (T2).

3.6. DAS-ELISA:

At the same time, the treated of faba bean seeds with rhizopium reduced significantly the concentration of BYMV in plants as proved by a significantly (P= 0.05) decrease in the absorbance values of ELISA reactions (Table 2).

Table 2. Valuation of plant growth improvement rhizobacteria – by inducing systemic resistance to BYMV in 45 – days old faba bean plants.

Treatments(1)	DI(2)	ELISA(3)	POA(4)
T1	0.00 d	0.08 c	0.13 c
T2	91.33 a	1.74 a	0.20 b
T3	17.27 c	0.55 bc	0.51 a
T4	22.06 b	0.60 b	0.48 a
$LSD_{0.05}$	1.913	0.5191	0.0998

(1) (T1): Negative control , (T2): Positive control (T3) : Inoculated with *Rhizobium leguminosarum* strain MNF-EM-R4 + infected with BYMV, (T4): Inoculated with *Rhizobium leguminosarum* bv.*viciae* strain MNF-EM-R9 + infected with BYMV.
(2) Disease incidence (%).
(3) ELISA absorbance reads, were measured at 405 nm.
(4) Peroxidase activity expressed as absorbance changes at 470 nm.

3.7. Determination of peroxidase enzyme activity:

The obtained results in table (2) showing a significant increase in (POA) in plants that inoculated with rhizobium compared to the negative and positive control. T3 (*Rhizobium leguminosarum* strain MNF-EM-R4)- inoculated plants showed a very high POA.

3.8. Antiviral protein (SDS-Page):

SDS-PAGE analysis was used to analysis the protein profiles using faba bean leaves (negative control, positive control, inoculated by *R. leguminosarum* strain MNF-EM-R4 and infected with BYMV and inoculated by *R. leguminosarum* bv. *viciae* strain MNF-EM-R9 and infected with BYMV virus. Fig (4) shows a considerable difference in protein profiles of all treatments with molecular mass from 15 kDa to 254 kDa. All treatments contain the main protein band between (21 to 128 kDa).The SDS-PAGE protein gel is analyzed using Clustering analysis using Jaccard's average (UPGAMA) clustering method, version (3.1). The zero-one (Table 3) was done for the gel according to the molecular mass of marker and existence of each band in the same molecular mass in all lanes of the gel. The zero one results showed that:

- Treatment with *R. leguminosarum* strain MNF-EM-R4 showed one band, this band did not appear in negative control , positive control and treatment with *R. leguminosarum* bv.viciae strain MNF-EM-R9 at molecular weight (40 kDa).
- Treatments with *R. leguminosarum* strain MNF-EM-R4 and treatment with *R. leguminosarum* bv.viciae strain MNF-EM-R9 showed one band did not appear in negative control, positive control treatments at molecular weight (85 kDa).

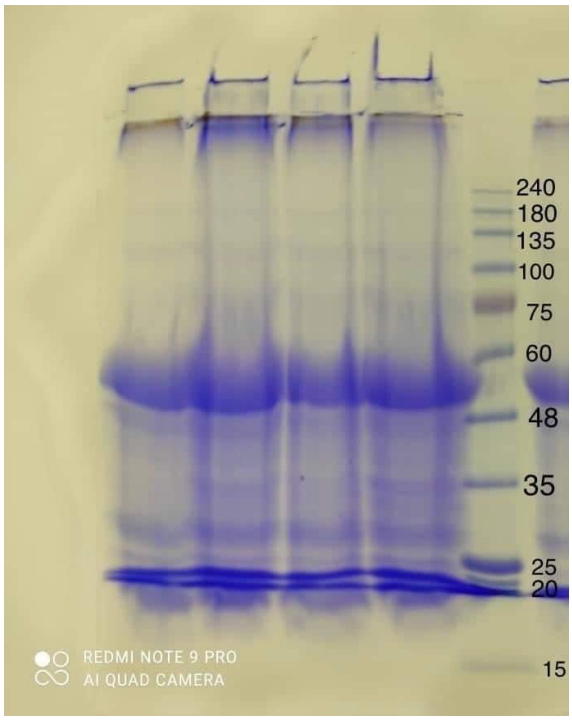

Fig (4): SDS-PAGE of total plant protein of all treatments: **M**: Protein marker (kDa). **1**: Negative control. **2**: Positive control **3**: Infected with BYMV+ Inoculated with *R. leguminosarum* strain MNF-EM-R4 **4:**Infected with BYMV+ Inoculated with *R. leguminosarum* bv.viciae strain MNF-EM-R9.

Table 3. Zero, one of negative control, positive control, BYMV+ *R.leguminosarum* (MNF-EM-R4) and BYMV+ *R.leguminosarum* (MNF-EM-R9).

Total MW	Negative control	Positive control	BYMV+ *R.leguminosarum* (MNF-EM-R4)	BYMV+ *R.leguminosarum* (MNF-EM-R9)
128	**1**	**1**	**1**	**1**
107	**1**	**1**	**1**	**1**
103	**1**	**1**	**1**	**1**
85	**0**	**0**	**1**	**1**
76	**1**	**1**	**1**	**1**
60	**1**	**1**	**1**	**1**
56	**1**	**1**	**1**	**1**
51	**1**	**1**	**1**	**1**
47	**1**	**1**	**1**	**1**
40	**0**	**0**	**1**	**0**
36	**1**	**1**	**1**	**1**
31	**1**	**1**	**1**	**1**
28	**1**	**1**	**1**	**1**
26	**1**	**1**	**1**	**1**
23	**1**	**1**	**1**	**1**
21	**1**	**1**	**1**	**1**

Discussion:

Under field conditions, BYMV severely affects various crops including faba bean plant (*Vicia faba* L.), this plant is among the most important economic legume crops grown in several areas of Egypt (Fegla *et al.*, 2003, Deya Eldeen, 2008 and Zeid, 2016) . In the present study, seven plant species from three various families, were tested for their sensitivity to infection by virus isolate. Described symptoms in leaves of Ch. *album*, *Ch. Quinoa*, *Vicia faba* L , *Phaseolus vulgaris* and *Pisum sativum* are nearly the same as described by (Sameh, 2005 and Deya El-deen *et al*., 2008) for bean yellow mosaic virus. Inoculations were further established by positive reactions to the antiserum against BYMV in DAS-ELISA test. But no visible symptoms were induced with *Nicotiana glutinosa* L (Hammed, 2006).

The isolate of BYMV was detected using serological tests (DAS-ELISA and Dot blot technique) which were positively used. Many investigators have used ELISA and Dot blot tests for serological detection of BYMV from different hosts (Kumar *et al*., 2015, Pradeep *et al*., 2015 , Zeid, 2016 and Hosny *et al*., 2021). TBIA approach has the advantage of being able to detect BYMV with a small amount of antigen compared to standard ELISA, as well as providing simplicity, speed, sensitivity, and convenience to large numbers of samples.

Light microsopy (L.M) is still essential in the study of histological abnormalities of tissue, and it can be used to check for the presence or absence of inclusion bodies (Matthews, 1991). Semithin sections of healthy and infected leaves were scanned after staining with fast green by light microscopy. The investigations revealed significant differences between healthy and infected tissues: the infected tissue's mesophyll layer was reduced and compacted, and the size of spongy and palisade cells was reduced, and the number of xylem arms and phloem layers was decrease compared to the healthy; similar findings were reported by others (Deya Eldeen *et al*., 2008).

Rhizobium species (PGPR) are widely used in agriculture for the advancement of legume crops due to its capability to fix atmospheric nitrogen in symbiosis with legumes. Improving faba bean plants by inoculation with rhizobia has been examined in the present work for suppression BYMV infection. This suppression induced through rhizobia may be direct by inhibition of plant growth or indirect by stimulating plant defense mechanisms (Kacem *et al*., 2009).

The first parameter used to assess the occurrence of SAR in faba bean plants treated with rhizobia was the reduction in BYMV percentage disease incidence as well as significant ELISA-value reduction. Our results are in harmony with those reported by (Rakib and Mustafa, 2013) who mentioned that BYMV infection caused a decrease in dry weight of shoot and root also, nodule number, whereas, inoculation with rhizobia induced significant increase in these parameters. Plants grown from seeds treated with Pseudomonas and Rhizobium bacteria showed a significant decrease in % disease incidence as well as a significant decrease in ELISA readings (virus concentration) as compared to untreated seeds with bacteria, according to Elbadry *et al*., (2006).

Systemic resistance promotion by rhizobacteria against Fusarium wilt in carnations seems to be due to bacterial lipopolysaccharides (LPS) (Van Peer and Schippers,1992). Furthermore, exopolysaccharides (EPS) and lipopolysaccharides (LPS) constitute the majority of Rhizobium cell surface carbohydrates. Polysaccharides play an important role in the symbiotic connection between legumes and Rhizobium throughout the recognition process (Denny, 1995).

The PGPR- suppression SR is frequently linked to the initiation of the defence mechanism, which includes the increased presence of defence enzymes such as peroxidase (Bergstrom *et al*., 1982). Increased peroxidase activity was recorded in Rhizobium-inoculated plants in our study. This result is in agreement with those of Elbadry *et al*., (2006) who mentioned that the degrees of PO activity were improved in faba bean leaves inoculated with Pseudomonas and Rhizobium bacteria. PO is one of the oxidative enzyme, play an great role in different physiological in plant specially metabolism and anabolism.It hydrolyzes hydrogenperoxide caused by dehydrogenase enzyme to water (Mengel, 1979).

The proteins of induced faba bean plants were examined using SDS-Page, and our findings revealed the formation of a novel pattern of proteins, as well as several increases in band density among biotic inducer treatments. The produced proteins may help to inhibit virus propagation or multiplications The proteins of induced faba bean plants were examined using SDS-Page, and our findings revealed the formation of a novel pattern of proteins, as well as several increases in band density among biotic inducer treatments. The produced proteins may help to inhibit virus propagation or multiplications (Mahmoud, 2000). Rakib and Moustafa (2012) mentioned that The polypeptide profile of viral particles obtained on the polyacrylamide gel electrophoresis, revealed a single protein of about 34 kd, representing the BYMV coat protein. A protein migrating at the similar speed in the profile of proteins from BYMV-infected plants was also identified; this protein being completely absent in the profile of uninfected plants. Electrophoretic tests by SDS-Page showed that foliar treatment with two plant extracts and their major components induced resistance against BYMV, therefore resulted in inducing new proteins, which were not established in healthy or infected and untreated plants. It has been recommended that, the induced proteins can help to limit virus infection or virus multiplication (Chen *et al*., 2006)

Conclusion

In conclusion, under greenhouse conditions, *R. leguminosarum* was isolated from faba bean roots and used as a promising inducer for SR in faba bean to BYMV infection. Because it is simple, cheap, and environmentally safe. This result could be a highly important strategy for controlling BYMV infection.

References

Bergstrom, G.C., Johnson, M. C. and Kuc, J.(1982). Effects of local infection of cucumber by *Colletotrichum lagenarium*, *Pseudomonas lachrymans* or tobacco necrosis virus on systemic resistance to cucumber mosaic virus. Phytopathology 72:922-926.

Bollag, D. M.; Edelstein, S. J. and Rozycki, M. D. (1996). Protein methods, 2nd edition, Wiley-Liss, New York, USA. 432pp

Chen, M., Qiu, D.W., Yang, X. F., Cao, K.Q. (2006). Inhibition of RNA replication and coat protein synthesis in Bean yellow mosaic virus by a plant activator protein. Chinese J. Biol. Control, 22(1):63-66.

Clark, M. F. and Adams, A. N. (1977). Characteristics of micro plate method of enzyme - linked immunosorbent assay for detection of plant viruses. Journal General of Virology, 34: 475- 483.

Dakora, F.D. (2003). Defining new roles for plant rhizobial molecules in sole and mixed plant cultures involving symbiotic legumes. New Phytologist. 158: 39-49.

Dakora, F.D., Joseph, C.M. and Phillips, C.M. (1993). Alfalfa root exudates contain isoflavanoids in presence of *Rhizobium meliloti*. Plant Pathology.101: 819-824.

Denny, T.P. (1995). Involvement of bacterial polysaccharides in plant pathogenesis. Annu. Rev. Phytopathol. 33, 173-197

Deya Eldeen, M. R., Guoquan, L., Khalaf, A. F. and Sabry, Y. M. (2008). Protective action of salicylic against *bean yellow mosaic virus* infection in *Vicia faba* leaves. Journal of Plant Physiology, 165(8): 845-857.

Efaisha, E.E.M. (2005). Studies on certain viruses affecting faba bean in El-beheira governorate [M. Sc. Thesis] Plant pathology Dept., Fac of Agriculture (Damanhour), Alex. Univ. p. 95.

El-Badry, M., Taha R.M. , Dougdoug K. A. and Gamal-Eldin, H. (2006). Induction of systemic resistance in faba bean (*Vicia faba* L.) to bean yellow mosaic virus potyvirus (BYMV) via seed bacterization with plant growth promoting Rhizobacteria. J. Plant Dis Port, 113(6): 247-251.

Enas, M. Abdel-Ghany , Ragaa, A. Eissa , Abdelmegid, I.Fahmi , Heaham, H. NaGaty and Abdelfatah, M. El-Zanaty (2020). Genetic characterization of some rhizobial isolates from various legumes. Journal of Biotechnology, computational Biology and Bionanotechnology 101(3) : 179-191.

Fegla, G.I. , Younes, H. A. , Abdelmonem A.M. and Rasmi, M.R.(2003). Incidence of some seed-borne viruses affecting faba bean in Alexandria Governorate. J adv Agric Res. 8:461-472.

Fehrman, H. and A. E. Dimond (1967). Peroxidase activity and phytophthora resistance in different organs of the potato. Plant Pathology,57:69-72.

Hamed, A. H. (2006). Studies on Plant Virus Inhibitors. M. Sc. Thesis, Faculty of Agriculture, Al-Azhar University, 133pp.

Hamed, A. H. , El-Banna, O. M., Ghanem, G.A.. , El-nagar, M. H. and Shafie, M. S. (2012). Isolation and identification of *Tobacco rattle Tobravirus* affecting onion (*Allium cepa* L.) plants in Egypt. International Journal of virology, 8 (1):39-49.

Hosny, A.Y., Mahmoud, H. A. and Ahmed, H. Zeid (2021). Detection of Bean yellow mosaic potyvirus in infected faba bean plants (*Vicia faba* L.) from Northern Egypt. Archieves of Phytopathology and Plant Protection 10:1-16.

Kacem, M., Kazouz, F., Merabet, C. A. and Bekki Abdelkader (2009). Antimicrobial activities of Rhizobium sp against *Pseudomonas savastanoi* the agent responsible for the olive knot disease in Algeria . Grasasy Aceites 60(2): 139-146.

Kumar, B., Talukdar, A., Verma, K., Bala, I., Harish, G. D., Gowda, S., Lal, S. K. and Sapra, R.L. (2015). Mapping of *Yellow mosaic virus* (YMV) resistance in soybean (*Glycine max* L. Merr.) through association mapping approach. Genetica, 143(1): 1-10.

Laemmli, U. K.(1970). Cleavage of structural proteins during the assembly of the head of bacteriophage.T4, Nature, 227 : 680-685.

Lin, N. S., Hsu, Y. H. and Hsu, H. T. (1990). Immunological detection of plant viruses and a mycoplasma like organism by direct tissue blotting on nitrocellulose membrans. Phtopathology, 80: 824 – 828.

Mahdy, A. M. M., Fawzy, R. N., Hafez, M. A., Mohamed Hanan .A.N. and Shahwan Eman. S. M.(2007). Inducing Systemic Resistance against *Bean yellow mosaic virus Potyvirus* Using Botanical Extracts. Egyptian Journal of Virology, 4: 223-240.

Mahmoud, S. Y.M. (2000). Antiviral substances induced in plants as a result of virus infection. Ph.D. Thesis. Faculty of Agriculture, Ain Shams University, Cairo, Egypt, 171 p.

Matthews, R. E. F. (1991). " Plant Virology" 3rd ed. Academic Press, Inc. 835pp.

Mclaughlin, M. R., Barnett, O.W. and Burrows, P. M. (1984). Enzyme – Linked immunosorbent assay of viruses infecting forage legumes. Phytopathology 74: 965-969.

Mengle, K. (1979). Influence of oxigenous factors on the quality and chemical composition of vegetables. Acta Horticulture, 93:133-151.

Mojca, M., Maja, R. and Maja, K. (2001). Peroxidases and photosynthetic pigments in susceptible potatoinfected with *Potato virus YNTN*. Plant Physiology and Biochemistry, 39 : 891-898.

Moury, B. and Desbiez, C. (2020). Host range evolution of potyviruses: A global phylogenetic analysis. Viruses. 12(1) : 111-120.

Pradeep, A., Jitendra, K. and Debjani, D. (2015). Evaluation of controlled release formulations of carbofuran against BYMV in soybean and their residues. Annals of Plant Protection Sciences, 23(1): 127-130.

Rakib, A. Al-Ani and Mustafa, A. Adhab (2013). Bean yellow mosaic virus (BYMV) on Broad bean: Characterization and Resistance induced by *Rhizobium leguminosarum* .J.Pure APPL MicroBio, 7(1) 135-142.

Reddy, D.V.R., Amin, P. W.,D. Mcdonald and A. M. Ghaneker, (1983). Epidemiology and control of groundnut bud necrosis and other diseases of legume crops in India caused by Tomato spotted wilt virus. In: R.T. Plumb , J. M. Thresh (eds): Plant virus Epidemiology: The spread and control of Insect-borne viruses pp. 93-102.

Robinson, G.H., Balk, J. and Domony, C.(2019). Improving pulse crops as a source of protein, starch and micronutrients. Nutr Bull. 44(3) : 202-215.

Ryals,J. S., Uknes, E. and Ward, A. (1994). Systemic acquired resistance. Plant Physiol. 104 : 1109-1112.

Sameh, K. H.(2005). Effect of *Bean yellow mosaic* virus on physiological parameters of *Vicia fabae* and *Phaseolus vulgaris*. International Journal of Agriculture and Biology, 7(2):154-157.

Van Loon, L. C. (1997). Induced resistance in plants and the role of pathogenesi-related proteins. European Journal Plants Pathology, 103:753-765.

Van Loon, L. C. Bakkers, P.A. and Pieterse, C.M.J. (1998). Systemic induced resistance by rhizosphere bacteria. Annu. Rev. Phytopathol. 36: 453-483.

Van Loon, L. C., Pierpoint, W. S., Boller, T. and Conejero, V.(1994). Recommendations for naming plant pathogenesis related proteins. Plant Molecular Biology Reporter, 12: 245-264.

Van Peer, R. and B. Schippers (1992). Lipopolysaccharides of plant growth promoting Pseudomonas spp. strain WCS417r induce resistance in carnation to Fusarium wilt. Neth. J. Plant Path. 98, 129-139

Ward, E. R., Uknes, S. J., Williams, S. C., Dincher, S. S., Wiederhold, D. L., Alexander, D. C., Ahl-Goy, P., Metraux, J. P. and Ryals, J .A. (1991). Coordinate gene activity in response to agents that induce

systemic acquired resistance. Plant Cell, 3: 1085-1094.

Whipps, J.M.(2004). Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Canadian Journal of Botany. 82 : 1198-1227.

Zeid, A. H. (2016). Biological and molecular studies on some viruses affecting faba bean [Ph Thesis]. Saba-Basha) Alexandria University. Faculty of Agriculture p. 136.

فاعلية بكتريا *Rhizobium leguminosarum* ضد فيروس موزايك الفاصولياء الأصفر علي نباتات الفول البلدي

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تم الحصول علي فيروس يسبب أعراض مختلفه (موزايك – تبقعات-التفاف اوراق-تشوهات) علي نباتات الفول البلدي في مناطق مختلفة في محافظة المنوفية- مصر . تم تعريف هذه العزلات بواسطة الأعراض-العوائل المشخصة- الأختبارات السيرولوجية(DAS-ELISA and DBIA) والميكروسكوب الضوئئ وكانت هذه العزلة لفيروس الموزايك الأصفر في الفاصوليا. أجريت الدراسة لتقييم كفاءة البكتريا العقدية*Leguminosarum Rhizobium* في تحفيز المقاومة الجهازية في نباتات الفول البلدي ضد الفايروس موزايك الفاصوليا الأصفر. وقد اظهرت النتائج أن النباتات الناتجة من هذه البذور المعاملة بالبكتريا انخفاض في نسبه حدوث الإصابة وكذلك تركيز الفيروس (قراءة الإليزا) . بينما أدت هذه المعملة الي زيادة في نشاط انزيم البيرواوكسيديز مقارنه بالكنترول المصاب والغير معامل بالبكتريا. أظهر تحليل البروتين باستخدام SDS-PAGE أن المعاملة ببكتريا *R. leguminosarum* strain with MNF-EM-R4 أظهرت حزمة بروتينيه عند وزن جزئئ (40 kDa) مقارنه بباقي المعاملات (السيمة والمصابه الغير معاملة بالبكتريا وكذلك المعاملة ببكتريا *R. leguminosarum* bv.viciae strain MNF-EM-R9 . وكذلك ظهرت حزمة بروتينية جديدة عند وزن جزئئ .(85 kDa) عند مقارنة ببكتريا *R. leguminosarum* bv.viciae strain MNF-EM-R9 and *R. leguminosarum* strain MNF-EM-R4 المعاملة بالكنترول السليم والمصاب الغير معامل بالبكتريا.