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Effect of Different Applications of Bio-agent *Achromobacter xylosoxidans* against *Meloidogyne incognita* and Gene Expression in Infected Eggplant

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Abstract

The present study aimed to evaluate the effect of different applications of the bio-agent *Achromobacter xylosoxidans* (B) against root-knot nematodes, *Meloidogyne incognita* (N) infected eggplant under greenhouse conditions. The different applications are addition of bacteria suspension seven days after / before nematodes, addition of bacteria suspension and nematodes at same time; root dipping in bacteria suspension for 30 and 45 min; foliar spreading by bacteria suspension, in addition two controls; infected plant with nematodes without applying any bacteria, and untreated plant neither with nematodes nor with bacteria. The best application of the bio-agent to maximize bio-control benefits was estimated by studying its effect on eggplant gene expression via protein analysis. The most effective application was when nematodes and *A. xylosoxidans* were added at the same time (N+B at same time). The reduction of nematode parameter; second-stage juveniles in the soil, number of galls and number of egg-masses due to this application had reached 79.80%, 71.09% and 78.26% respectively. These results are in harmony with those of soluble protein electrophoresis of eggplants, which showed that the infected plant gave the lowest number of bands in all total stages of the experiment, while the application (N+B at same time) had the highest number of bands. Four bands at M.W. (55, 53, 47 and 43 kDa.) produced by untreated plant and all application but were absent in infected plant. This indicates the importance of applying the bacterium strain *A. xylosoxidans* which induced the plant to produce these important proteins. The increase or decrease in the number of protein bands refers to the induction or inhibition of the resistance genes, and this change was reflected on plant growth. The effectiveness of the application was clear in increasing the length, fresh and dry weights of shoot and root.

Keywords: Different applications, *Achromobacter xylosoxidans*, eggplant, *Meloidogyne incognita*, bio-agent, gene expression, SDS-PAGE.

1. Introduction

Plant-parasitic nematodes cause serious losses to a variety of agricultural crops worldwide (Ismail *et al.*, 2018). Egyptian agriculture faces a great loss every year incurred from infection by plant diseases, the annual losses up to 23% in eggplant (El-Nagdi *et al.* 2019). The root-knot nematodes, *Meloidogyne incognita* are major plant-parasites, and they are the main problem for many agricultural crops (Hussain *et al*., 2012; Youssef *et al*., 2012; Mukhtar *et al*., 2013, Kassab *et al*., 2017). In recent years, the main way for their control was the use of chemical nematicides. The risk and harmful effects on humans and environments have focused the attention on the development of biological control agents as an alternative potential eco-friendly strategy for controlling plant diseases. Soil rhizobacteria are repeatedly shown to be promising microorganisms for the biological control of plant-parasitic nematodes (Giannakou *et al.,* 2004). The use of biological agents in particular plant growth-promoting rhizobacteria (PGPR) has been considered as an attractive viable option to the control strategies (Kloepper *et al.,* 1991*).* B Due totheir ability to improve plant growth, Endophytic bacteria can be considered as PGPR, and their close interactions with plants make them an ideal candidate for enhancing plant growth.

Endophytic bacteria, *Achromobacter xylosoxidans* is a gram-negative bacterium, frequently- isolated from the rhizosphere. *A. xylosoxidans* use in biocontrol is already known to suppress nematode population (Yuen and Schroth, 1986; Vaidya *et al.,* 2001; Tian *et al.,* 2007). *A. xylosoxidans* was included as plant growth -promoting rhizobacteria (PGPR) as it has shown tremendous promise in terms of improvement of NO_3 uptake by roots. *A. xylosoxidans* strain SF2 produces salicylic acids, and another endophytic *A. xylosoxidans* strain 31A was reported to tolerate salinity (Sgroy *et al.,* 2009). Siderophores of *A. xylosoxidans* can act in biocontrol as a determinant of induced systemic resistance in the plant (Vaidya *et al.,* 2001, Forchetti *et al*., 2007). Also, *A. xylosoxidans* Ax10 has solubilized inorganic phosphate and *A. xylosoxidans* Ax10 has the capability of producing indole acetic acid. In pot experiments, inoculation of *A. xylosoxidans* Ax10 significantly led to an increase of the root length, shoot length, fresh weight, and dry weight of *Brassica juncea* plants compared to the control (*Ma et al.,* 2009). Zhang *et al.* (2016) found that the culture filtrate of

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two bacterial strains *A. xylosoxidans* (09X01) and *Bacillus cereus* (09B18) caused high mortality of the second stage juvenile nematodes and reduced *in vitro* egg hatch compared to control.

Using the bacterial suspension simultaneously with nematodes and/or before nematode inoculation was more effective than adding after nematode inoculation. In addition, it recorded a significant increasing in nematode and plant parameters compared to the infected plant with nematodes (Soliman *et al*., 2017).

Polyacrylamide gel electrophoresis (SDS-PAGE) is commonly employed in biological analysis to determine shifts in protein bands. These bands might be proteins or enzymes. Bio-stress, due to hormonal changes, could cause protein synthesis and enzymatic shifts (Ghasempour *et al*. 1998; Gianello *et al.* 2000. Ghasempour *et al*. 2001; Ghasempour and Kianian 2002; Ghasempour and Maleki 2003). The amount of soluble protein increases in desiccation-hardened plants and undergoes changes in electrophoretic mobility (Faw and Jung, 1972). Cloutier, (1983). detected quantitative changes in the electrophoretic patterns of the soluble proteins of different cultivars grown in different environments. Certain types of stressful environmental conditions can activate stress genes to produce stress proteins that enable organisms to tolerate such stresses. The electrophoretic banding patterns can be used for characterization and identification of eggplant cultivars (Sayed *et al.* 1998). Electrophoretic patterns of soluble proteins and isozymes are powerful tools for the study of genetic variability of cultivars (Barta *et al.*, 2003). Vyomesh *et al* (2018) detected increasing number of bands in both cultivars upon transition from control to stress environment, and resistant cultivar showed more number of bands as compared to susceptible cultivar

The present study aimed to evaluate the efficacy of different application of *A. xylosoxidans* as bio-agent to maximize its efficiency against *M. incognita* infecting eggplant under greenhouse conditions. Also, this research attempted to study the effect of some different applications on eggplant gene expression via soluble protein analysis.

2. Materials and methods

The bacterial bio-agents *Achromobacter xylosoxidans* 19GES registered under accession number LC214968.1 in the GeneBank (USA) was obtained from previous studies and cultured on Luria broth medium at 30° C. Luria broth (LB) medium is prepared from Tryptone 10 g, Yeast Extract 5 g, NaCl 5 g, 20 g up to 1000 ml of distilled water according to Davis *et al.* (1980).

2.1. Preparation of Nematode, M. incognita culture

The nematode population used in this research, *M. incognita*, was obtained from greenhouse culture of tomato plants which were maintained at Plant Pathology Department. The nematode eggs were extracted from infected roots of tomato in 0.5 % sodium hypochlorite on 25 µm sieve according to the method of Hussey and Barker (1973). The eggs were incubated at 27 ºC inside incubator and hatched out the second- stage juveniles (J_2) by using Baermann plates.

A. xylosoxidans liquid culture was prepared and activated in (LB) medium. Plastic pots (15 cm diameter) filled with two kilograms of autoclaved soil, 1:1 mixture of clay and sand, were prepared, and then the eggplant seedlings were transplanted. Pots were arranged in a randomized complete block design under greenhouse conditions at 28°C±2. All plants were watered after nematode inoculation and whenever needed. Six applications of A. *xylosoxidans* were applied in the soil:

The bacterial culture *A. xylosoxidans* was applied as soil drench application (a, b, c), barer root dipping (d and e) and foliar spreading as bio-agent to bio-control of *M. incognita* as follows:

a. *A. xylosoxidans* (B) inoculated seven days after the nematodes (N) inoculation (B after 7days N).
b. Nematodes (N) added after seven days from the *A. xylosoxidans* (B) inoculation (N after 7days B).
c. The nematodes (N) and *A. xylosoxidans* (B) inoculated simultaneously (N+B at same time).
d. Barer root dipping treatment was given to seedling by immersing their roots in the *A. xylosoxidans* suspension for 30 minutes (Root dipping at 30 min.).
e. Barer root dipping treatment was given to seedling by immersing their roots in the *A. xylosoxidans* suspension for 45 minutes (Root dipping at 45 min.).
f. Foliar spreading of leaves plant (Foliar spreading with bacteria, *A. xylosoxidans*).
g. Plant with nematodes (Infected plant).
h. Plant without bacteria and nematodes (Untreated plant).

Applications of rhizobacterial bio-agents were performed by adding 1 ml of *A. xylosoxidans* (around 2×10^8 cfu / ml) in 2 cm deep around each plant and covered with soil of a seedling at the time of transplant.

Barer root dipping treatment was given to seedling by immersing their roots in the culture of bacteria (2×10^8 cfu /ml) for two times, 30 min and 45 minutes.

Nematode suspension of *M. incognita* (2000 J_2) was added to soil, where seedlings were planted in 2 cm deep around each plant and covered with soil. Plants infected with *M. incognita* and untreated with bacteria served as control. All pots were arranged in a randomized complete block design in a greenhouse at 28 ± 2 ºC. Fifty days after nematode inoculation, eggplants were gently uprooted, and the roots were washed and cleaned from the adhering soil particles. The second- stage juveniles (J_2) in the soil were extracted by sieving and decanting technique (Barker, 1985) and examined under a light microscope using a Hawksley counting slide. Number of J_2 in soil, number of galls and number of egg masses were counted from the whole root system and indexed according to Sharma *et al.* (1994) scale as follows: 1 = no galls or egg-mass, 2 = 1 - 5, 3 = 6 - 10, 4 = 11 - 20, 5 = 21 - 30, 6 = 31 - 50, 7 = 51 - 70, 8 = 71 - 100 and 9 >100 galls or egg-mass / plant. Lengths, fresh and dry weights of both shoot and root systems were recorded.

2.2. SDS-Protein Electrophoresis

Samples of 1 g from leaves exposed to different applications of *A. xylosoxidans* as bio-agent against *M. incognita* infecting eggplant greenhouse conditions in different times were used for protein analyses by Sodium Dodecyl Sulfate –Polyacrylamide Gel Electrophoresis (SDS-PAGE). The protein analysis was preformed according to the method of Laemmli (1970). Samples preparations and extraction of water-soluble proteins were performed according to Stegmann (1979). The gel was

photographed and scanned by Gel Doe Bio-Rad System (Gel- Pro analyzer V.3).

2.3. Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) and significant means separated with Duncan Multiple Range Test (DMRT) at $P \leq 0.05$ levels according to Duncan (1955).

3. Results

3.1. Effect of different applications of bio-agent Achromobacter xylosoxidans against root-knot nematodes M. incognita number of J2, galls, and egg-masses formation.

Effect of different applications of *A. xylosoxidans* as bio- control agent against *M. incognita* infected eggplant under greenhouse conditions is shown in Table (1). The results indicated that all application of *A. xylosoxidans* culture had achieved significant reduction ($P<0.05$) on root-knot nematode parameters as compared to untreated plant. *A. xylosoxidans* (N+B at same time) application was the most effective as reduction reached 79.80%, 71.09% and 78.26% on number of second-stage juveniles in soil, number of galls and number of egg-masses respectively. Application of nematodes after *A. xylosoxidans* (N after 7days B) comes next with some exceptions, recording 75.76%, 68.38% and 75.35% reduction on number of J_2, number of galls and number of egg-masses, respectively,while the least effective application was in case of barer root dipping, where immersing of seedling root for 30 min. recorded 49.90%, 54.38% reduction in the number of J_2 and number of galls, respectively. Also, the same trend was noticed with respect to galls and egg-masses indexes.

Table 1. Effect of different applications of bio-agent *Achromobacter xylosoxidans* against root-knot nematodes *M. incognita* number of J_2, galls, and egg-masses formation (after eight weeks applications). #

Treatment	No. J_2 200 g in soil	R%.	No. galls / root system	R%.	Root gall index**	No. egg-masses / root system	R%.	Egg-mass index**
B after 7day N	128*c	74.14	51.00b	47.96	7	5.33c	76.83	3
N after 7day B	120d	75.76	31.33de	68.38	6	5.67c	75.35	3
N+B adding the same time	100e	79.80	28.33de	71.09	5	5.00c	78.26	2
Root dipping at 30	248b	49.90	45.00bc	54.08	6	7.00bc	69.57	3
Root dipping at 45 min	124cd	74.95	36.33cd	62.93	6	9.33b	59.43	3
Leaf spraying	123d	75.16	39.67cd	59.52	6	6.67bc	71.00	3
Infected plant	495a	----	98.00a	------	8	23.00a	------	5

Values are average of five replicates. * Means followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test. ** Root galls and egg-masses indexes were determined. R. % = % Reduction.

3.2. Effect of different applications on bio-control efficacy of A. xylosoxidans against root-knot nematodes, M. incognita on the growth traits of infecting eggplant.

The results shown in Table (2) indicated that the influence of all different applications of *A. xylosoxidans* had increased growth parameters of eggplant significantly ($P<0.05$). The data revealed that application of *A. xylosoxidans* (N+B at the same time) had achieved the most effective application in increasing the length, fresh and dry weights of shoot and root length where they reached 58.57 %,120.30 %, 130.26 %, 93.33 %, compared to other applications and infected plant (plant +nematodes). Generally, application of *A. xylosoxidans* in soil drenching and foliar spraying appeared more effective in increasing growth of eggplants compared to immersing of the seedling roots.

Table (2): Effect of different applications on bio-control efficacy of *Achromobacter xylosoxidans* against root knot nematode *Meloidogyne incognita* on the growth traits of infecting eggplant (after eight weeks of applications).#

Treatments	Shoot System						Root System			
	Length (cm)	% Inc.*	fresh weight(g)	% Inc.	Dry weight(g)	% Inc.	Length (cm)	% Inc.	fresh weight(g)	% Inc.
B after 7 days N	24.25*b	38.57	3.13bc	54.95	1.19b	56.58	14.75ab	51.28	2.06bc	19.84-
N after 7days B	26.00 ab	48.57	3.39b	22.28	1.31b	72.37	12.75bc	30.77	2.12bc	-17.51
N+B adding in the same time	27.75a	58.57	4.45a	120.30	1.75a	130.26	18.85a	93.33	2.03c	-21.10
Root dipping at 30 min	18.25c	4.23	3.26b	61.39	1.14bc	50.00	12.75bc	30.77	2.38ab	7.39-
Root dipping at 45 min	20.00c	14.29	2.47c	22.28	1.17bc	53.95	17.75a	82.05	2.23bc	-13.23
Leaf spraying	24.00b	37.14	2.47c	22.28	1.46ab	92.11	16.25ab	66.67	2. 33ab	9.39-
Untreated plant	19.00	8.57	3.53		1.50		11.25	15.38	2.18 bc	-15.18
Infected plant	17.50c		2.02c		0.76c		9.75c		2.57a	

Values are average of five replicates. * Means followed by the same letter (s) are not significantly ($P \leq 0.05$) different according to Duncan's Multiple Range Test. % Inc* = Increase over control.

3.3. Effect of A. xylosoxidans applications on infected eggplant gene expression by SDS-PAGE:

3.3.1. After one week

Protein profile was performed to detect the biochemical differences due to the different applications of *A. xylosoxidans* as a bio-agent against *M. incognita* which infects eggplant in greenhouse conditions compared with untreated plants and infected plants as shown in Figure (1) and Table (3). The results revealed clear differences in the number and molecular weights of protein bands, 15 bands were found ranging from 17 to 145 kDa. were polymorphic (100% polymorphism). The highest number of protein bands was found in the (root dipping 45 min.) application (12 bands), followed by (B after7days N) application (11 bands) while the lowest number of bands was observed in the infected plant (4 bands) . In addition, the application of *A. xylosoxidans* (N+B at same time) displayed eight protein bands. One band with M.W. 145 kDa. appeared in the infected plant but was absent in untreated plant and the rest of applications. On the contrary, two bands at 39 and 24 kDa. were found in untreated plant, leaf spraying and root dipping 45 min. applications, but absent in the infected plant. Also, four bands at M.W. 55, 53, 47and 43 kDa were produced by untreated plant in normal conditions and all applications but were absent in infected plant.

Table 3: Densitometric analysis represents leaf water soluble-protein electrophoretic patterns for eggplant under normal and different applications after one week.

B.N	M.W.	Untreated plant	**Infected plant**	B after 7 days N	N after 7 days B	**N+B at same time**	Root dipping 30 min	Root dipping 45 min	Leaf spraying
1	145	0	1	0	0	0	0	0	0
2	78	0	0	1	0	0	0	0	0
3	67	1	0	1	1	1	1	1	1
4	62	0	0	1	0	0	0	0	0
5	55	1	0	1	1	1	1	1	1
6	53	1	0	1	1	1	1	1	1
7	47	1	0	1	1	1	1	1	1
8	43	1	0	1	1	1	1	1	1
9	39	0	0	0	0	0	0	1	1
10	31	1	0	1	0	0	0	1	0
11	29	1	1	1	0	1	0	1	1
12	26	1	1	1	0	1	1	1	1
13	24	0	0	0	0	0	0	1	1
14	20	1	1	1	0	1	1	1	0
15	17	1	0	0	0	0	0	1	0
Number of Bands		10	4	11	5	8	7	12	9

1 = Presence of band 0 = absence of band B,N = Band Number M.W. =Molecular weight

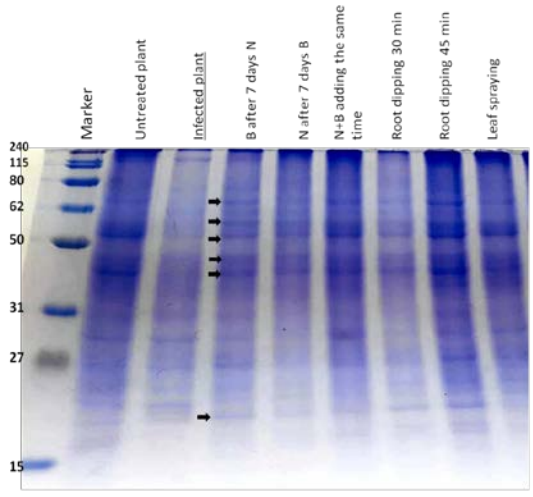

Figure 1. SDS-PAGE of leaf water-soluble protein electrophoretic patterns for eggplant under normal and different applications after one week.

3.3.2. after two weeks of application

Figure (2) and table (4) showed the protein profile of different applications compared with the infected plant (plants + nematode). A total of 12 bands were found in protein pattern ranging from 140 to 19 kDa. Only one band was monomorphic (8.33%) while eleven were polymorphic (91.67% polymorphism). The highest number of protein bands was 11 bands observed in the application of *A. xylosoxidans* (N+B at same time), three of them at M.W. (140, 27 and 21 kDa. respectively) were not detected in untreated plant and the rest of applications, followed by (B after 7days N) application (six bands). The lowest number of bands (two bands) was observed in the infected plant (plant + nematode). The exposure of plants to stress during root dipping for 45 min. in the bacterial suspension prior to transplantation caused weakness of plants sample, which made it impossible to take sample leaves for protein electrophoresis. We expect that exposure of the plants to this period of time 45 min. is too much, so the bacterial suspension does reverse action to the plants.

Table 4. Densitometric analysis represents leaf water-soluble protein electrophoretic patterns for eggplant under normal and different applications after two-week application.

B.N	M.W	Infected plant	B after 7 days N	N after 7 days B	N+B adding the same time	Root dipping 30 min	Leaf spraying	Untreated plant
1	140	0	0	0	1	0	0	0
2	80	0	1	1	0	0	0	0
3	50	0	1	1	1	1	1	1
4	46	0	1	1	1	1	1	1
5	43	0	1	1	1	0	0	1
6	31	1	1	1	1	1	1	1
7	33	1	0	0	1	0	0	0
8	27	0	0	0	1	0	0	0
9	23	0	0	0	1	0	1	1
10	22	0	1	0	1	0	0	0
11	21	0	0	0	1	0	0	0
12	19	0	0	0	1	0	0	1
Number of Bands		**2**	**6**	**5**	**11**	**3**	**4**	**6**

1 = Presence of band 0 = absence of band B,N = Band Number M.W. =Molecular weight

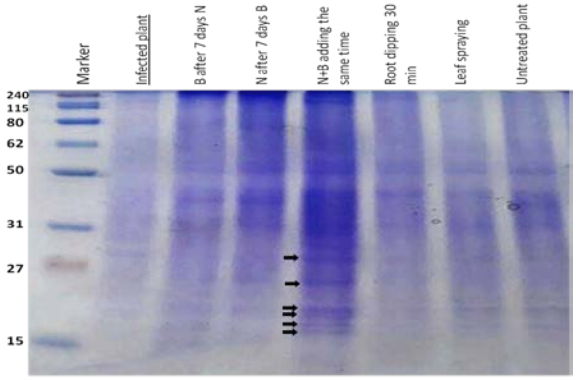

Figure 2. SDS-PAGE of leaf water soluble protein electrophoresis patterns for eggplant under normal and different application after two weeks

3.3.3. End of the experiment (after eight weeks)

Electrophoresis patterns for eggplant under normal and different applications at the end of experiment are shown in Figure (3) and Table (5). The results showed that most of the extracted proteins migrated in the range from 151 to 22 kDa. with total number of 10 bands were polymorphic (100% polymorphism). The highest number of protein polypeptides was found in (N+B at same time) application (7 bands), while the lowest number of bands was observed in the (infected plant) and (root dipping 45 min. application) one band.

Table 5. Densitometric analysis represents leaf water-soluble protein electrophoretic patterns for one eggplant variety under normal and different applications after eight- weeks of experiment.

B.N	**M.W**	Infected plant	B after 7 days N	N after 7 days B	N+B adding the same time	Root dipping 30 min	Root dipping 45 min	Leaf spraying	Untreated plant
1	**151**	1	0	0	0	0	0	0	0
2	**57**	0	1	1	1	1	1	1	1
3	**56**	0	0	0	1	0	0	0	0
4	**44**	0	0	1	1	1	1	0	1
5	**42**	0	0	0	1	1	0	0	0
6	**41**	0	0	1	0	1	1	0	1
7	**29**	1	0	0	0	0	0	0	0
8	**25**	0	0	1	1	1	1	0	1
9	**23**	0	0	0	0	1	0	0	0
10	**22**	1	0	1	0	1	1	0	1
Number of Bands		**3**	**1**	**5**	**5**	**7**	**5**	**1**	**5**

1 = Presence of band 0 = absence of band B,N = Band Number M.W. =Molecular weight

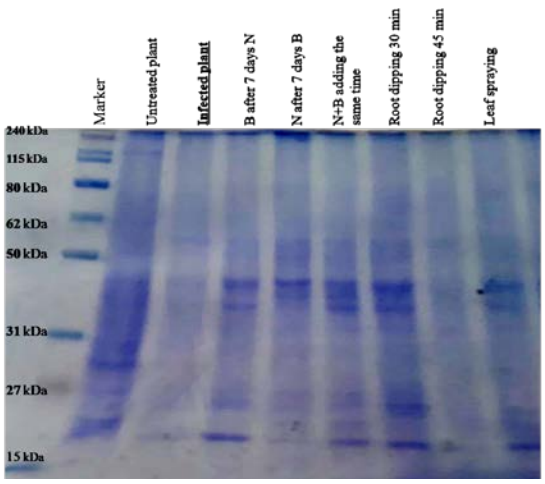

Figure 3. SDS-PAGE of leaf water soluble-protein electrophoresis patterns for one eggplant variety under normal and different application after eight- week of the experiment**.**

4. Discussion

Plant parasitic nematodes, root-knot nematode (*Meloidogyne incognita*), is one of the most important nematodes associated with low production (Kayani *et al*., 2017).

Biological control of plant- parasitic nematodes through microorganisms offers an alternative or supplemental management tool to replace chemical methods. Use of biological control agents is considered to be innocuous and economically feasible (Mukhtar *et al*., 2017). *A. xylosoxidans* can be considered as a potential biocontrol agent to control plant pathogens and improvement of plant growth. These results agreed with those Vaidya *et al.* (2001), Forchetti *et al.* (2007) and Zhang *et al.* (2016). Moreover, Zhang *et al*. (2016) found that the culture filtrate of *A. xylosoxidans* (09X01) strain caused high mortality of the second stage juvenile nematodes and reduced in vitro egg hatch compared to control. The present data are in agreement with Soliman *et al.* (2017) who reported that applying bacterial suspensions simultaneously with nematodes and or before nematode inoculation was more effective in reducing nematodes infection than in case of adding after nematode inoculation compared to the infected plant with nematodes and without bacteria**.**

The application of *A. xylosoxidans* (N+B at the same time) had more effective application in increasing the length, fresh and dry weights of shoot and root length compared to other applications and infected plant (plant +nematodes). The present data agreed with Khan and Tarannum (1999) who reported that root dip treatment was relatively less effective in enhancing the plant growth and yield of tomato compared to soil application. This effect was due to a higher and more uniform distribution of bacterial cells in the root zone during the entire growth period, whereas with root dip treatment, fewer cells may have remained available to the entire root -system. Soliman *et al*. (2017) found the soil where bacterial suspension was applied simultaneously with nematodes and or before nematode inoculation was more effective than adding the bacterial suspension after nematode inoculation. The used applications recorded a significant increase in the shoot and root lengths and weights when compared to untreated control. The results of soluble protein electrophoresis indicated that the infected plant (plant + nematodes) gave the lowest number of bands in all stages of applications (9 bands). Four bands at M.W. 55, 53, 47 and 43 kDa. Were produced by untreated plant in normal conditions and all application but were absent from infected plant, which indicates the negative effect of nematodes on the infected plant and its inability to produce important proteins for its growth and development in the absence of the bacterium bio-agent. This indicates the importance of applying the bacterium strain *A. xylosoxidans* which helped the plant to produce these important proteins, and the effectiveness of the application was clear in increasing the length, fresh and dry weights of shoot and root length which had high records more than records with infected plant. On the other hand, the application of *A. xylosoxidans* (N+B at same time) showed the highest number of bands in all total stages of experiment (26 bands), three of them at M.W. (140, 27, 21 kDa. respectively) were unique bands and appeared after two weeks but were not detected in untreated plant and the rest of applications. We suggested these bands may be related to gene resistance in plant. The results of electrophoresis coincided with those of greenhouse experiments where bacteria (*A. xylosoxidans*) strain was applied as a bio-agent against nematodes infecting eggplant. The application of *A. xylosoxidans* was generally effective and had achieved reduction of the number of second-stage juveniles in the soil, gall number and number of egg-masses. In addition, this application gave clear increasing in shoot and root lengths and fresh and dry weights compared with the infected plant. The treatment of *A. xylosoxidans* (N+B at the same time) was the most effective application which achieved reduction of 79.80%, 71.09% and 78.26% in the number of second-stage juveniles in the soil, galls number and number of egg-masses, respectively. Moreover, the data revealed that the application of *A. xylosoxidans* (N+B at the same time) achieved clear increasing in length, fresh and dry weights of shoot and root length were recorded 58.57%,120.30 %, 130.26% and 93.33%, comparedg with the infected (plant + nematodes).

Finally, the increase or decrease in number of protein bands refers to the induction or inhibition of the resistance genes and this change was reflected on plant growth. This result agreed with results obtained by (Faw and Jung, 1972 and Cloutier 1983) who found that the amount of soluble protein increases in desiccation-hardened plants and undergoes changes in electrophoretic mobility detected quantitative changes in the electrophoretic patterns of the soluble proteins of different cultivars grown in different environments; and agreed with those of (Ghasempour and Maleki 2003). The bands obtained might be proteins or enzymes. Bio-stress, due to hormonal changes could cause protein synthesis and enzymatic shifts. The result agreed with those of Vyomesh *et al* 2018 who detected increasing number of bands in both the cultivars upon transition from control to stress environment and resistant cultivar showed more number of bands as compare to susceptible cultivar

5. Conclusion

In conclusion, the best application of the bacterium strain *A. xylosoxidans* when added with the nematode *Meloidogyne incognita* at the same time, followed by add nematodes (N) after seven days from the *A. xylosoxidans* (B) inoculation, (N after 7days B). This application improved its efficiency as a bio-agent compared to other applications, and could be used as a safe biological alternative to chemical nematicides for suppressing root knot nematode reproduction and improving plant growth parameters.It was found that the best time to study protein bands which produce under nematodes stress and application was after one and two weeks.

Practically, the authors recommend applying the bio-agent *A. xylosoxidans* as soil drench at the time of planting or transfer of plant for protection against *Meloidogyne incognita* infection.

Reference

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