JJBS

Volume 16, Number 2,June 2023
ISSN 1995-6673
Pages 233 – 242
https://doi.org/10.54319/jjbs/160207

Jordan Journal of Biological Sciences

Bioefficacy of *Bacillus cereus* and its Three Mutants by UV Irradiation Against *Meloidogyne incognita* and Gene Expression in Infected Tomato Plants

Gazeia M. Soliman[1], Sameh M. El-Sawy[2], Rasha G.Salim[3], Ghada M. El-Sayed[3] and Walaa A. Ramadan[4,*]

[1]*Plant Pathology Department, Nematology Unit, National Research Centre, Dokki, Giza, Egypt;* [2]*Vegetable Research Department, National Research Centre, Dokki, Giza, Egypt;* [3]*Microbial Genetics Department, National Research Centre, Dokki, Giza, Egypt;* [4]*Genetics and Cytology Department, National Research Centre, Dokki, Giza, Egypt.*

Received: June 10, 2022; Revised: November 5, 2022; Accepted: November 25, 2022

Abstract

Stress the use of safer options such as biological control to avoid the hazards of chemical nematicides. UV irradiation has been used to induce mutations and improve protease overproduction. All mutants recorded significant decreases in nematode-related parameters when compared with the wild type and control. Mutant number one (M1) achieved high protease activity about 2.5 fold. At two concentrations of the wild type *Bacillus cereus* and their mutants effect on percentage mortality of *Meloidogyne incognita* juveniles. M1 achieves the highest mortality, 99.33 %, and achieved greatest reductions in the number of juveniles in soil, juveniles/5 g root, galls, and egg-masses recorded 89.76 %, 90.16 %, 85.29 %, and 89.11 %, respectively. M1 achieved the best increase of the growth parameters of plant length, number of branches, number of leaves, and fresh weight of leaves recorded 54.35 %, 71.45 %, 169.44 %, and 256.17 %, respectively. These results are in harmony with those of leaves water soluble-protein electrophoretic patterns after one month and the end of the application, which showed that the plant exposed to M1 exhibited the highest number of bands in both stages of application and appeared unique band at M.W 55kDa after one month of application.

Keywords: Biological-control, *Bacillus cereus*, Mutation, UV irradiation, *Meloidogyne incognita*, SDS-protein electrophoresis, Tomato plants

1. Introduction

The world's human population is rapidly expanding, and as a result, food consumption is rising. Because of the rising demand for food, producers have turned to new techniques to produce more food in the same cultivation area. Tomato (*Solanum lycopersicum* L.) is regarded as one of Egypt's most important vegetable crops. The root-knot nematodes are one of the highly important pathogens that cause great damage and losses incurred to horticultural and field crops, and they are found all over the world; some species are found in tropical and subtropical areas of Africa, such as Egypt **(**Ismail *et al.,* 2018). These losses ranged between 24 and 38 % as a result of root-knot nematode infestation (El-Nagdi *et al.* 2019; Netscher and Sikora 1990).

Root-knot nematodes, *Meloidogyne* spp., have a diverse host range, attacking up to 5500 plant species (Trudgill and Blok, 2001). The traditional method of nematode management through the use of chemical nematicides is more effective, but it has drawbacks such as high costs and risks to human health and the environment (Mitiku, 2018; Migunova and Sasanelli, 2021). Concerns about public health and environmental safety are driving calls to reduce chemical nematicides used in plant-parasitic nematode control. As a result, immediate changes in chemical control are required, as is encouraging scientists to look for natural compounds that are less toxic and more environmentally friendly. Biological control by plant-growth-promoting rhizobacteria is a safe alternative approach and promising tool for controlling plant-parasitic nematodes (Kumar and Arthurs, 2021; Lee and Kim, 2016; Rika *et al*., 2017; Priyank *et al*., 2018; Sidhu, 2018; Soliman *et al*., 2020).

Several rhizosphere bacteria have been identified as having potential antagonistic activity against plant-parasitic nematodes by producing enzymes. Protease, chitinase, and lipase are among the lytic enzymes secreted by bacteria. For tylenchoid nematodes like *Meloidogyne* spp., the primary reasoning for their usage in plant nematodes control originates from the fact that the biochemical makeup of nematode structure comprises collagens and lipids during mobile phases, as well as protein, chitin and lipids during sedentary stages (Bird and McClure, 1976). In *in vitro* testing, when compared to the control, *B. cereus* culture filtrates has a nematicidal effect on *M. incognita* egg hatching and mortality (Soliman *et al*., 2019). Extracellular enzymes, including proteases, are produced by *B. cereus* (Shumi *et al*., 2004; Soliman *et al*., 2019). *B. cereus* strain supernatant was used to treat *M. incognita*, which resulted in strong nematicidal activity

* Corresponding author. e-mail: walaanrc70@yahoo.com.

and 90.96 % death. *B. cereus* strain can create extracellular chemicals that kill nematodes (Gao *et al*., 2016; Mohamed *et al*., 2021). Eissa *et al*. (2010) used ultraviolet irradiation to increase the nematicidal effect of the chitinolytic bacterium, the wild type of *B. thuringiensis* against *M. incognita,* infecting sunflower. When compared to the wild type and the untreated control, the mutants employed reduced the number of juveniles, galls, and egg-masses while improving plant development parameters.

Usually, bacteria produce valuable products, such as enzymes, in the required amounts to benefit; thus, they tend not to overproduce their metabolites (Shafique *et al*., 2021). A successful relationship between mutational genetics and industrial microbiology has been reported. Many studies have been published on the use of the strain improvement process through mutations to produce various industrial enzymes such as chitinase, lipase, cellulase, glucoamylase, and protease (Eissa *et a.l*, 2010; Ismail *et al*., 2019; Rakib, *et al*., 2020). Mutation techniques are used to improve the biocontrol agents to manage phytopathogens. Physical and chemical mutagens have been applied by many researchers to generate new biotypes (Rey *et al*., 2000). Ultraviolet irradiation (UV) was used to improve the enzyme activity of the original strains as a protease (Afifi *et al*., 2014; Wang *et al.*, 2007).

Proteins represent the final product of gene expression in organisms; protein isolation and characterization have been one of the most important subjects for researchers. The biochemical markers which study the protein expression make more exact identification possible. The biochemical techniques are rapid, accurate, and dependable (Sammour, 2014). Many reports show that the protein pattern changes are accompanied by biological changes in the process of adaptation, making the organism more fit in the altered environment (Hurkman, *et al*., 1998; Singh *et al*., 1985). Polyacrylamide gel electrophoresis SDS-PAGE is commonly used in biological analysis to identify shifts in protein bands. These bands might be enzymes or proteins. Bio-stress, due to hormonal changes, could cause protein synthesis and enzymatic shifts (Ghasempour *et al*., 2001; Ghasempour and Kianian, 2002; Ghasempour and Maleki, 2003). Increasing bands were detected in both cultivars upon transition from control to stress environment and the resistant cultivar showed more bands as compared to the susceptible cultivar (Vyomesh *et al.,* 2018; Ramadan and Soilman, 2020).

2. Material and Methods

2.1. Bacterial strain

The bacterial strain of *Bacillus cereus* NRC215, isolated and identified in the microbial genetic department, recorded under accession number MT229271 in the NCBI database, was used in this investigation and grown in Luria Broth medium (LB) according to (Davis et al., 1980).

2.2. Genetic Improvement via Ultraviolet irradiation (UV) mutagenesis

One milliliter of eighteen hours old bacterial culture *B. cereus* (saturated culture), as wild type was re-suspended in 50 ml LB medium-containing flasks for 4 h to ensure that cells were in exponential phase and that according to (Alireza 2016). For UV treatment, 10 ml of saturated culture were centrifuged washed twice, and re-suspended in an equal volume of 50 mM phosphate buffer, pH 7.0, and 2-ml sample was evenly placed in a sterile glass petri dish (5 cm in diameter). One plate was kept in dark which served as control and the rest were exposed to UV-light (A 30-w germicidal lamp at 254 nm (VL-130.G, Vilber, Germany) from a distance of 20 cm for 3, 5, and 10 min. After treatment, samples were immediately diluted 1:10 in LB medium-containing flasks wrapped in tin foil and grown to saturation. Then, appropriate cell dilutions from treated and control samples were spread on LB plates with and without RIF (100 µg/ml) to isolate induced and spontaneous RIF's resistant mutation, respectively.

2.3. Protease assay

Petri dishes 150 mm diameter each contains 50 ml of 0.4% gelatin medium were inoculated from the 24–hours age slants of the evaluated *B. cereus* and its mutants by UV irradiation. Inoculated plates were then incubated for 48 hours at 30 ºC after which 7-10 ml of the test solution (100 ml Dist. Water + 15 g. $HgCl_2$ + 20 ml HCl) were pipetted on the surface of agar. Relationship between diameter of analysis zone and the bacterial growth zone, then, the difference between them divided by the diameter of the growth zone was calculated according to Smith *et al.*, (1952).

2.4. Preparation of Nematode, M. incognita culture

M. incognita, the nematode population employed in the bioassay experiment, was obtained from the Plant Pathology Department's greenhouse culture of tomato plants. The eggs were incubated at 27°C in an incubator and hatched out to second-stage juveniles (J2) using modified Baermann plates, which were counted under a stereoscopic microscope.

2.5. Identification of nematode from field area

Roots infected with root-knot nematode were collected from the research field. The perineal pattern of mature females was used to identify root- knot nematode species (Eisenback1985).

2.6. Nematocidial activity of B. cereus wild type and its mutants by UV irradiation on percentage mortality of M. incognita juvenile's in vitro bioassay

Effect of wild types and different mutants of lytic *B. cereus (*by UV irradiation) on percentage mortality of *M. incognita* juveniles**,** Two concentrations (100 and 50 %) from cell suspension of wild types (pre-genetic improvement bacteria) and 3 mutants from *B. cereus* were prepared by sterile water. 1 ml of freshly hatching juvenile suspension (50 juveniles±5 / 1 ml) with 2 ml of culture filtrate were transferred to test type. Juveniles that kept in 3 ml of distill water served as control. Five replicates of each treatment were used. The test types were incubated at room temperature (28± 2º C). The juveniles which did not regain their activities and do not move when probed with fine needle were considered "dead". On the other hand, the juveniles were considered active when they were visibly flexible. Handling tools were cleaned with sterilized water throughout the experiment. The percentages of juvenile survival were calculated under a microscope. The percentages of nematode mortality were calculated according to Abbott's Formula as follows:

Juvenile mortality (%) = (m – n)/ (100 – n) × 100, where m and n indicate the percentages of mortality in treatments and control, respectively.

2.7. Effect on M. incognita developmental parameters:

The initial population of *M. incognita* were estimated in field experiments before transplanting time by taking five samples of about 200 g soil subsamples of well-mixed soil collected from each row. Then, sieving and decanting nematodes were extracted from this soil by Barker (1985). Four months after transplanting, soil and root samples were collected and the numbers of juveniles (J_2) of *M. incognita* in soil were counted using Hawksly slide under light microscope. Root samples were gently washed free of soil and an aliquot of 5 g per plot (5 plants) was cut into 2-cm-long pieces. At each sampling time, the root pieces were placed in Petri dishes with distilled water incubated under laboratory conditions (25 ± 5 ºC) for a week to extract and count *M. incognita* J2. The numbers of nematode galls and egg-masses were also counted in another 5 g root. The percentages of nematode reduction in total nematode stages inside the roots J2, galls and egg-masses on roots per 5g were calculated with respect to untreated control. J2 in soil were calculated according to the formula of Henderson and Tilton (Puntener, 1981). Gall Index Measuring: The gall index of *Meloidogyne* infested roots was measured as described by Zeck, (1971) on a scale from 0-10.

2.8. Field experiment

Suppressive effect of wild type of *B. cereus* and its three mutants against *M. incognita* infected tomato plant was carried out at the Experimental and Production Station of National Research Centre, El-Noubaria region, Beheira Governorate, north of Egypt, during 2020 and 2021 season, to study the effect wild type of *B. cereus* and its three mutants (M1, M2, M3) on combination on growth, chemical composition, flowering, yield and fruit quality of tomato (*S. lycopersicum* L.) cv. CH7, tomato seedlings were transplanted to the field experiment at (September). The experiment was arranged in a randomized complete block design. The plot area was 10.5m2 (3 x 3.5m) with five rows.

All agricultural practices for this plant were carried out as recommended by the Ministry of Agric., Egypt All of the treatments that were evaluated were used on the soil drench. The bacteria were applied by rate 100 ml / around plant root at two doses (1ml contains about 10^{12} cfu) after 3 and 30 days from transplanting date. Vydate was applied with a rate of 0.2 ml/plant, and 12.5 kg/fed, as recommended rates in Egypt by Ali and El-Ashry (2021). This experiment included the following treatments:

1- Untreated plants (Control).
2. Vydate® 24% L (Oxamyl)
3. Mutant 1(M1)
4. Mutant2 (M2)
5. Mutant3 (M3)

Five plants were randomly chosen from each replicate at 65 days from transplanting date to determine the following parameters:

2.8.1. Vegetative growth characteristics

Plant length (cm, number of leaves per plant, number of branches per plant and Fresh and dry weights of leaves per plant (g).

2.8.2. Flowering characteristics

Number of clusters per plant.

2.8.3. Fruit yield

Tomato fruits were hand harvested when reaching red ripe stage. Total marketable yield was calculated considering red and disease-free fruits. Number of fruits per plant, fruit yield per plant (g) and total marketable yield (ton/fed) were calculated.

2.8.4. Fruit quality

Random samples of fruits were taken from each experimental plot at the middle of harvesting stage to determine the following characteristics

2.8.5. Fruit physical quality characteristics

Average fruit weight (g) and Average diameter (cm)

2.8.6. Fruit chemical quality characteristics

Determination of total soluble solids (TSS %): Total soluble solids were measured in harvested fruits using hand refractometer, (Atago, U.S.A.).

2.9. SDS-Protein Electrophoresis

Samples of 1 g from leaves exposed to different applications were used to detect the induction of systemic resistance (ISR) in tomato plants infected with *M. incognita* via quantitative and determination of the soluble and non-soluble proteins. This method was done according to Laemmli (1970). Sample preparation and extraction of water-soluble and non-soluble2 proteins were performed according to Stegmann (1979). The gel was photographed and scanned by Gel Doe Bio-Rad System (Gel- Pro analyzer V.3). SDS-PAGE was as modified by Studier (1973).

2.10. Statistical analysis

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

3. Results

3.1. Genetic improvement of B. cereus protease by UV irradiation

UV treatment of *B. cereus* for three minutes resulted from six rifampin-resistant mutants. There were no rifampin-resistant mutants after five and ten minutes. Only three of the six mutants have lytic activity and have generated protease, and their nematicidal activity against *M. incognita* has been tested under laboratory and field conditions.

3.2. Protease production

The protease activities of *B. cereus* and its three mutants were assessed using gelatin agar, and the diameter of the clear zone was measured. When compared to other mutants and the wild type, the results showed that mutant1 (M1) achieved high protease 250% about 2.5-fold followed by M3 and M2. Generally, the mutants produced

the maximum protease activity compared with the wild type Figure 1(A and B)

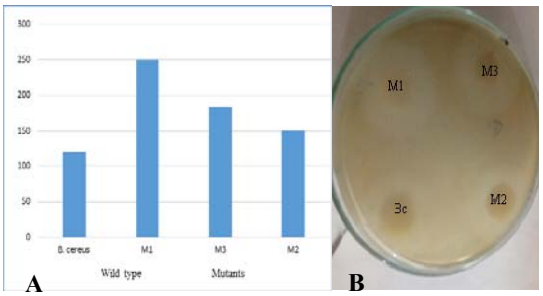

Figure 1. A: Protease activity of *B. cereus* and its three mutants M1, M2, and M3. B: Clear zone of protease activity of *B. cereus* and its three mutants M1, M2, and M3.

3.3. Effects of B. cereus and its three mutants on M. incognita J2 mortality

The results in (Table1) indicated that the effect of bacterial filtrate of *B. cereus* and its mutants on percentages mortality of *M. incognita* juveniles after 24 h at two concentrations 100 % and 50%. allowed to recover by their transferred to aerated water indicated that mortality percentages after 24 h significantly ($P < 0.05$) increased at using all mutants as compared to the wild type and control. Their percentages of mortality ranged from 99.33 % at M1 and 92.00% at using M3 compared to 87% when applied the wild type at the first concentration.

In the second concentration, percentages of mortality ranged from 96.33 % at M1 to 89.33 % at using M3 compared to 82 % at wild type. Also, there was a positive relationship between the nematode mortality and concentrations.

Also, there was a positive relationship between the nematode mortality and the protease production from the mutants and the wild type (Table 1).

Table 1. Effect of wild type of *B. cereus* and its three mutants by UV irradiation on percentage mortality of *M. incognita* juveniles after 24h.

Concentrations / Treatments	Nematode Mortality			
	Wild type	Mutants		
	B. cereus	M 1	M2	M 3
100 %	87.00*d	99.33a	95.00b	92.00c
50%	82.00c	96.33a	91.66b	89.33b

* Means followed by the same letter(s) are not significantly ($P \leq 0.05$) different according to Duncan's Multiple Range Test

3.4. Nematicidal potential of B. cereus and its three mutants against M. incognita infecting tomato plants

Table 2 shows the effect of B. cereus and M1, M 2, and M3 on nematode parameters as compared to vydate and untreated plants (control). The tested wild type and mutant were more effective than the control at reducing the number of M. incognita J2 in soil, no. J2 in 5 g root, galls, and egg-masses /5 g root. The M1 treatment achieved the greatest reductions in the number of J2 in the soil J2 in the 5 g root, galls a number, and a number of egg-masses, with reductions of 89.76 %, 90.16 %, 85.29 %, and 89.11 %, respectively. It is followed by M2 with 78.40 %, 89.31 %, 81.51 %, and 86.14 % J2 in the soil, J2 in g root, galls number, and number of egg-masses, respectively. While the wild type had a reduction of 43.45 and, 26.56 &, 62.18 %, and 65.94 % in the number of second-stage juveniles in the soil when compared to the untreated control, J2 had a reduction of 5 g root, galls, and egg-masses when compared to the untreated control.

Table 2. Effect of B. cereus wild type and its mutants on development of M. incognita parameters infected tomato plant under field conditions.

Treatments		Nematode parameters									
		No. J_2 in soil (250g)	% R	No. J_2 / 5 g root	% R	No. galls / 5 g root	% R	Root gall index**	No. egg-masses / 5 g root	% R	Egg-masses index**
Untreated plant	Control	475.33*a	--	315.00 a	---	79.33a	---	8	67.33a	---	7
Vydate	Nematicides	201.67c	50.75	214.33b	31.96	28.33 b	64.29	5	15.33c	77.23	4
B. cereus	Wild type	268.33b	43.45	231.33b	26.56	30.00b	62.18	5	23.00b	65.94	5
M 1	Mutants	48.67e	89.76	31.00d	90.16	11.67d	85.29	4	7.33e	89.11	3
M2		102.67d	78.40	33.67d	89.31	14.67d	81.51	4	9.33de	86.14	3
M 3		112.67d	76.30	102.00c	67.61	20.67c	73.94	4	11.33d	83.17	4

*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 according to Zeck (1971) 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 . R % = % Reduction*

3.5. The potential effect of B. cereus and its three mutants on improving tomato plants characteristics.

3.5.1. Vegetative growth of tomato plants

The effect of *B. cereus* and M1, M 2, and M3 compared to the vydate and untreated plant (control) on vegetative growth of tomato plants grown in sandy soil was shown in (Table 3). The results revealed that tomato plants treated with M1 and M2 had the highest significant of plant length which increased by 54.35% and 47.22% respectively compared to the untreated plants, with non-significant differences between the two mutants. In the same trend,

M1 and M2 treatments produced the highest significance of number of branches per plant compared to the other treatments and increased by 71.45% than the control treatment. On the other hand, tomato plants treated by M1 had the maximum significant of number of leaves and fresh weight of leaves per plant, where the values increased by 169.44% and 256.17%, respectively, compared to the control plants.

3.5.2. Flowering and fruit yield of tomato plants

Regarding the effect of *B. cereus*, M1, M2, and M3, vydate and control treatments on flowering and fruit yield of tomato plants, data in Table 3 illustrated that M1 and M2 treatments outperformed the other studied treatments with significant differences, where the number of clusters and number of fruits per plant increased by 188.51% and 185.60% and by 107.09% and 94.14% with M1 and M2 treatments, respectively, while the M1 treatment only achieved the highest significant for fruit per plant and fruit yield (ton/ fedden) with increasing by 35.49% and 35.48%, respectively, compared to the control treatment.

Table 3. Effect of wild type of B. cereus and its three mutants by UV irradiation on vegetative growth of tomato plants#.

Treatments		Plant length (cm)	% Increase	Number of branches/ plant	% Increase	Number of leaves/ plant	% Increase	Fresh weight of leaves/ plant (g)	% Increase
Untreated plant	Control	108.00* e	---	2.33 c	----	36.00 f	---	226.80 f	----
Vydate	Nematicides	135.00 d	25.00	3.33 b	42.86	70.33 e	95.36	402.70 e	77.56
B. cereus	Wild type	143.00 cd	32.41	3.33 b	42.86	79.67 d	121.31	496.90 d	119.09
M1	Mutants	166.70 a	54.35	4.00 a	71.45	103.00 a	186.11	904.20 a	298.68
M 2		159.00 ab	47.22	4.00 a	71.45	97.00 b	169.44	807.80 b	256.17
M 3		150.00 bc	38.89	3.67 ab	57.18	90.67 c	151.86	689.50 c	204.01

*Means followed by the same letter(s) are not significantly (P≤0.05) different according to Duncan's Multiple Range Test. # Values are average of five replicates

3.5.3. Fruit quality of tomato plant

Regarding the effect of *B. cereus*, M1, M2, and M3, vydate and control treatments on the fruit quality of tomatoes, data in Table 4 showed that the highest significant values of tomatoes average fruit weight and fruit diameter were noticed with M1 and M2 treatments with non-significant differences between them. The results show that tomato plants treated by M1 and M2 treatments increased the mentioned characteristics by 135.55% and 126.77% and by 24.81% and 24.09%, respectively. On the other hand, there were no significant differences among *B. cereus*, M1, M2 and M3 treatments with superiority upon vydate and control treatments.

Table 4. Effect of wild type of *B. cereus* and its three mutants by UV irradiation on flowering and fruit yield of tomato plants.

Treatments		Number of clusters/ plant	% Increase	Number of fruits/ plant	% Increase	Fruit yield (g/plant)	% Increase	Fruit yield (ton/ fedden)	% Increase
Untreated Plant	Control	11.67 d		28.33 d		1905.00 d		28.58 d	
Vydate	Nematicides	17.00 c	45.67	36.00 c	27.04	2122.00 c	11.39	31.83 c	11.37
B .cereus	Wild type	18.33 c	57.07	34.33 cd	21.18	2217.00 c	16.38	33.26 c	16.37
M1	Mutants	33.67 a	188.51	58.67 a	107.09	2581.00 a	35.49	38.72 a	35.48
M 2		33.33 a	185.60	55.00 a	94.14	2426.00 b	27.35	36.40 b	27.36
M2 3		26.67 b	128.53	44.33 b	56.48	2351.00 b	23.41	35.27 b	23.41

Table 5. Effect of wild type of *B. cereus* and its three mutants by UV irradiation on fruit quality parameters of tomato plants.

Treatments		Average fruit weight (g)	% Increase	Fruit diameter (cm)	% Increase	TSS %	% Increase
Untreated plant	Control	59.18 e		4.57 d		3.70 c	
Vydate	Nematicides	86.93 d	46.89	5.00 c	9.48	4.72 b	27.49
B. cereus	Wild type	98.20 c	65.93	5.13 c	12.39	5.17 a	39.65
M 1	Mutants	139.40 a	135.55	5.70 a	24.81	5.00 ab	35.16
M 2		134.20 ab	126.77	5.67 a	24.09	5.00 ab	35.16
M 3		126.10 b	113.08	5.40 b	18.24	5.00 ab	35.14

3.6. Effect of wild type and different mutants of B. cereus, on Protein expression in infected tomato plants

3.6.1. after one month of application

Protein profile (soluble proteins and non-soluble proteins) was performed to detect the biochemical differences in infected tomato plants. The results revealed clear differences in the number and molecular weights of protein bands due to the different applications of wild type and different mutants of *B. cereus* as a bio-agent against *M. incognita*.

The results of water soluble proteins appeared in Table (6) and figure (2) the electrophoresis pattern showed 10 bands with molecular weight 12-140 kDa, four bands were monomorphic at M.W 62, 29, 17 and 12kDa while six bands were polymorphic. The infected plant exposed to mutant 1 exhibited the highest number of bands (10 bands) including unique band at M.W 55kDa. The lowest number four 4 bands appeared in infected plant exposed to wild type of *B. cereus.*

Table 6. Densitometric analysis represents leaves water soluble protein electrophoretic patterns for infected tomato plant under normal and different applications after one month.

B.N	M.W	Untreated plant	Vydate	Wild type of *B. cereus*	*Mutant 1*	*Mutant 2*	*Mutant 3*
1	140kDa	+	+	-	+	+	+
2	68kDa	+	+	-	+	+	+
3	62kDa	+	+	+	+	+	+
4	55kDa	-	-	-	+	-	-
5	50kDa	+	+	-	+	-	+
6	40kDa	-	-	-	+	-	+
7	32kDa	+	+	-	+	+	+
8	29kDa	+	+	+	+	+	+
9	27kDa	+	+	+	+	+	+
10	12kDa	+	+	+	+	+	+
Total Bands		8	8	4	10	7	9

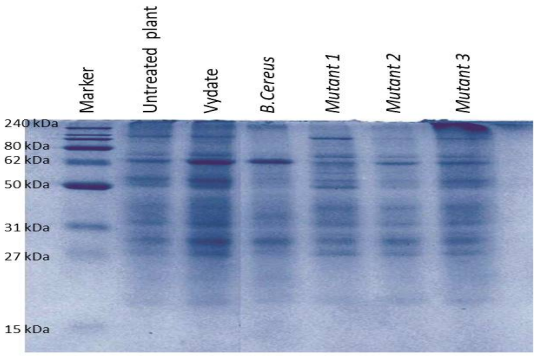

Figure 2. SDS-PAGE of leaves water soluble protein electrophoresis patterns for infected tomato plant under normal and different after one month.

Water non-soluble proteins presented in table (7) and finger (3) a total of seven bands were found in protein pattern ranging from 18 - 62 kDa, two bands were monomorphic while five bands were polymorphic. The lowest number was two bands appeared in the infected plant that were exposed to the wild type of *B. cereus.*

Table 7. Densitometric analysis represents leaves water non-soluble protein electrophoretic patterns for infected tomato plant under normal and different applications after one month

B.N	M.W	Untreated plant	Vydate	Wild type of *B. cereus*	*Mutant 1*	*Mutant 2*	*Mutant 3*
1	62 kDa	+	+	+	+	+	+
2	53 KDa	+	+	+	+	+	+
3	45KDa	+	+	-	+	+	+
4	31KDa	+	+	-	-	-	-
5	21KDa	+	+	-	+	+	+
6	20KDa	+	+	-	+	+	-
7	18KDa	+	+	-	+	-	-
Total Bands		7	7	2	6	6	4

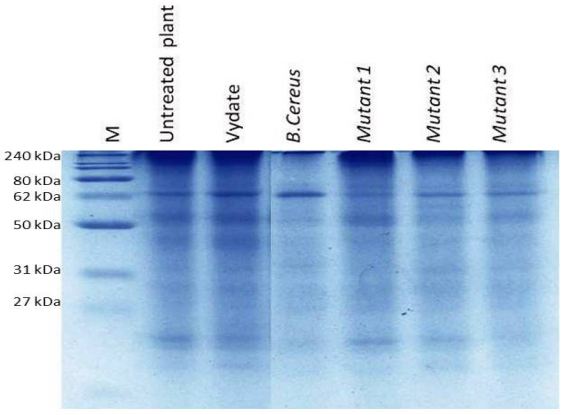

Figure 3. SDS-PAGE of leaves water non-soluble protein electrophoresis patterns for infected tomato plant under normal and different applications after one month.

3.6.2. End of experiment

The results of water soluble proteins of end of experiment presented in Table (8) and figure (4). Showed 13 bands with MW ranging from 18 – 240 kDa, with; seven bands were monomorphic while six bands were polymorphic. The highest number of bands (12 bands) appeared in plants exposed to M1 of *B. cereus.*

Table 8. Densitometric analysis represents leaves water soluble protein electrophoretic patterns for infected tomato plant under normal and different applications at end of experiment.

B.N	M.W	Untreated plant	Vydate	Wild type of *B. cereus*	*Mutant 1*	*Mutant 2*	*Mutant 3*
1	240kDa	-	-	-	-	+	-
2	90 kDa	+	+	+	+	+	-
3	62 kDa	+	+	+	+	+	-
4	57kDa	+	+	+	+	+	+
5	52kDa	+	+	+	+	+	+
6	45kDa	+	+	+	+	+	+
7	39kDa	+	+	+	+	+	+
8	32kDa	+	+	+	+	+	+
9	27kDa	+	+	+	+	+	+
10	25kDa	+	+	+	+	-	+
11	22kDa	-	-	-	+	-	-
12	20kDa	+	+	+	+	+	+
13	18kDa	+	+	+	+	-	+
Total Bands		11	11	11	12	9	9

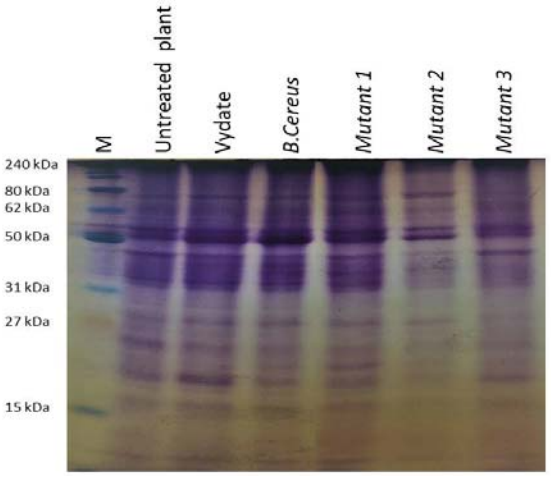

Figure 4. SDS-PAGE of leaves water soluble protein electrophoresis patterns for infected tomato plant under normal and different applications at end of experiment.

Water non-soluble proteins Table (9) and figure 5 showed that a total of nine bands were found in protein pattern ranging from 29 – 115 kDa, eight bands were monomorphic while one band was polymorphic. Band at MW 115kDa appeared only in plants exposed to M1and M3 of *B. cereus.*

Table 9. Densitometric analysis represents leaves water non-soluble protein electrophoretic patterns for infected tomato plant under normal and different applications at end of experiment.

B.N	M.W	Untreated plant	Vydate	Wild type of *B. cereus*	*Mutant 1*	*Mutant 2*	*Mutant 3*
1	115kDa	-	-	-	+	-	+
2	65kDa	+	+	+	+	+	+
3	62kDa	+	+	+	+	+	+
4	59kDa	+	+	+	+	+	+
5	51kDa	+	+	+	+	+	+
6	35kDa	+	+	+	+	+	+
7	31kDa	+	+	+	+	+	+
8	30kDa	+	+	+	+	+	+
9	29kDa	+	+	+	+	+	+
Total Bands		8	8	8	9	8	9

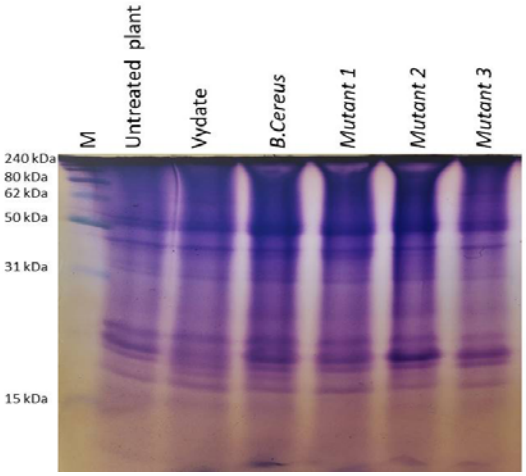

Figure 5. SDS-PAGE of leaves water non-soluble protein electrophoresis patterns for infected tomato plant under normal and different applications at end of experiment

4. Discussion

Plant-parasitic nematodes infect a wide range of agricultural crops around the world, causing significant losses. The root-knot nematode, *M. incognita* is the most common nematode related to low yield (Kayani *et al*., 2017). Synthetic nematicides pose a risk to humans and the environment, emphasizing the importance of instruments like biological control in sustainable agricultural systems (Giannakou *et al.,* 2004). The use of biological control agents is considered to be safe (Mukhtar *et al*., 2017; Soliman *et al*., 2020). The antagonistic microorganisms, particularly those that produce lytic enzymes, are one of the most promising alternatives to chemical nematicides (El-Alfy and Schlenk 2002; Ashoub and Amara 2010; Kashyap *et al*., 2022). Bacteria produced lytic enzymes when they come into contact with parasitic nematode, which helps to degrade the eggshell, prevent egg hatching, and/or inhibit juvenile activity (Xiang *et al*., 2018). Biological control with rhizosphere bacteria has been shown to improve plant growth (Kashyap *et al*., 2021) and reduce nematode reproduction through a variety of mechanisms including increased nitrogen-fixing ability and plant growth hormones production (Mohamed *et al*., 2021; Saharan and Nehra 2011).

These enzymes have an important role in bacterial-nematode-plant interactions and could be used as a nematicidal to keep nematode populations suppression in the soil (Lian *et al*., 2007). UV mutation was used to induce the generation of a protease-overproducing mutant from *B. cereus*, according to (Afifi *et al*., 2014). In our result, when compared to other mutants and the wild type, mutant M1 had a 2.5-fold increase in protease. UV irradiation is being to increase protease overproduction by inducing mutations agreement (Afifi *et al.,* 2014).

Mutation achieved increasing in alkaline protease-over producing mutants, which produced two to three times more activity than the wild type of *S. marcescens* (Kassab *et al*., 2017). There were positive relationships between the nematode mortality and each of the bacteria concentration and enzyme production from the mutants in bioassay; this result agrees with Kassab *et al*. (2017). The wild type of *B. cereus* and its mutants with their two concentrations show stronger nematicidal activity against second-stage juveniles (J2) of *M. incognita*, according to the results of the laboratory experiment. These results agreed with those of (Eissa *et al*., 2010; Kassab *et al*., 2017). All mutants recorded significant ($P < 0.05$) decreased nematode-related parameters and increased growth parameters of the plant when compared with the wild type and control this results in agreement (Kassab *et al*., 2017; Ismail *et al*., 2018).

Quantitative proteins of induced tomato plants infected with nematode were identified by using SDS- PAGE; our results indicated that the plant exposed to M1 exhibited the highest number of soluble protein bands (10 bands) including a unique band at M.W 55kDa, after one month of application, also gave The highest number of soluble protein bands (12 bands) at the end of the application. These results are in harmony with those of plants exposed to M1 products, which significantly increased all plan growth parameters and tomato yield. This indicates the importance of applying the M 1 of bacterium strain B. cereus, which helped the plant to produce increasing protein bands.

An increase in the number of protein bands refers to the induction of the resistance genes, and this change was reflected in plant growth. This result agreed with those of (Chen et al., 2006; El-Dougdoug et al., 2014; Sofy et al., 2014). They suggested that the induced proteins may help to limit the spread or multiplication of pathogens. Sharaf *et al*., (2016) detected a new pattern of proteins, with different increase in the density of bands when they used antagonistic bacterial strains of *B. subtilis* against the root-knot nematode M. incognita infecting tomato plants.

Our results indicated that presence of leaves soluble protein bands with molecular weights 27and 32 KD (PR3 –Chitinase) in all plants treated with biotic inducers. Chitin is a major cell wall component of many bacteria, fungi and nematodes. Chitinase can hydrolyze the cell walls of the pathogen (Hassan, 2004; Kashyap *et al*., 2020). Several investigators have indicated that induced resistance in plants has been associated with a significant increase in chitinase activity. These results agreed with those of Sharaf *et al*. (2016) who found that the presence of molecular weight 27and 32 KD (PR3 –Chitinase) of protein bands on all plants treated with biotic inducers; these induced proteins have been defined as pathogenesis-related proteins, and they are implicated in plant defense due to their anti-pathogenic activities (Van-Loon *et al.,* 1994).

The outcomes of this study demonstrate that *B. cereus* and its mutations can be employed as an alternative to nematicides in the biological control of *M. incognita*, which results in improved tomato quality and quantity as well as a contribution to the creation of more sustainable agriculture.

5. Conclusion

B. cereus and its mutations can be used as an alternate nematicide to manage *M. incognita* biologically. As a result, all mutants had significantly lower nematode-related parameters as compared to the wild type and control. The best mutant application was Mutant M1, which resulted in the highest juvenile mortality in soil, and achieved a reduction in juveniles in root, galls and egg-masses and improving the plant growth parameters. Also the plant who exposed to M1 exhibited the highest number of protein bands in both stages of application

Reference

Abbot WS. 1925. A method for computing the effectiveness of insecticides. *J. Econ. Ent.*,**18**: 265-267.

Afifi AF, Abo-Elmagd HI, Housseiny MM. 2014. Improvement of alkaline protease production by *Penicillium chrysogenum* NRRL 792 through physical and chemical mutation, optimization, characterization and genetic variation between mutant and wild-type strains. *Ann Microbiol.*, **64**:521–530. doi:10.1007/s13213-013-0685-y

Ali AAI and El-Ashry RM. 2021. Potential effect of the nematicide oxamyl and surfactant combinations on root-knot nematode *Meloidogyne incognita* infecting tomato plants *Egypt Acad J Biolog Sci.,* **13(1):**159-176

Alireza G. 2016. UV-Induced Mutagenesis in Lactic Acid Bacteria. *J. Genet. Genom*., **4**: 1-4, doi: 10.11648/j.ijgg.20160401.11.

Ashoub AH and Amara MT. 2010. Biocontrol activity of some bacterial genera against root-knot nematode, *Meloidogyne incognita*. *.J AmrSci*., **6**: 321-328.

Barker KR. 1985. Ne.matode extraction and bioassay In: An advanced treatise on *Meloidogyne*, Volume 11- Methodology (Eds K. R. Barker, C. C. Carter and J. N. Sasser)., **19**- 35. North Carolina State University Graphics: Raleigh. North Carolina, USA.

Bird A and McClure M. 1976. The tylenchid (nematode) egg shell: structure, composition and permeability. *Paracitology.*, **72**:19-28.

Chen M, Qiu DW, Liu Z, Yang XF and Cao KQ. 2006. Inhibition of RNA replication and coat protein synthesis in Tobacco mosaic virus by a plant activator protein. *Chinese Journal of Biological Control.*, **22:** 63-66. (English abstract).

Davis RW, Botstein D, Rotho JR .1980. Transfection of DNA. In bacterial genetics: a manual for genetic engineering advanced bacterial genetic, vol **67**. Cold Spring Harbor laboratory cold spring harbor, *New York.*, 134–137.

Duncan DB. 1955. Multiple range and multiple F tests. *Biometrics*., **11**: 1-42.

Eisenback JD. 1985. Detailed Morphology and Anatomy of Second-Stage Juveniles, Males and Females of the Genus *Meloidogyne* (Root-Knot Nematodes). In: Advanced Treatise on *Meloidogyne*, Sasser, J.N. and C.C. Carter (Eds.). Vol. 1, North Carolina State University Graphics, Raleigh., 47-77.

Eissa MFM, Ismail AE, Abd- El Bary NA, Yassin MY, Bader UM, Soliman GM. 2010. Control of *Meloidogyne incognita* using a wild type and its three mutants of the chitinolytic bacteria, *Bacillus thuringiensis* under greenhouse conditions. *Bull. NRC.*, **35 (2**): 139-149.

El-Alfy A and Schlenk D. 2002. Effect of 17{beta}-estradiol and testosterone on the expression of flavin-containing mono oxygenase and the toxicity of aldicarbto Japanese Medaka Oryziaslatipes. *Toxicol Sci.*, **68**: 381–388.

El-Dougdoug KA, Sofy AR, Mousa AA and Refaey EE. 2014. Monitoring variability responses of cultivated potato varieties infected with Potato virus Y pepper isolate. *Egyptian J of Virology.*, **11(2)**: 82-101.

El-Nagdi MAW, Abd-El-Khair H, Soliman GM, Ameen HH and El-Sayed M G. 2019.Application of protoplast fusants of *Bacillus licheniformis* and *Pseudomonas aeruginosa* on *Meloidogyne incognita* in tomato and eggplant. *Middle East J of Appl Sci.,* **9 (2)**: 622-629.

Gao GH, Qi G Yin R, Zhang H, Li C and Zhao X. 2016. *Bacillus cereus* strain S2 shows high nematicidal activity against *Meloidogyne incognita* by producing sphingosine. *Scientific Reports.*, **6**:28756 1-11. DOI: 10.1038/srep28756.

Giannakou IO, Karpouzas DG, Prophetou-Athanasiadou D. 2004. A novel non-chemical nematicide for the control of root-knot nematodes. *Appl. Soil Ecol*., **26**:69–79.

Ghasempour HR and Kianian J. 2002. Drought stress induction of free proline, total proteins, soluble sugars and its protein profile in drought tolerant grass *Sporobolus elongates. J. Sci. Teacher Training Univ.*, **1**: 111-118 .

Ghasempour HR and Maleki M. 2003. A survey comparing desiccation tolerance in resurrection plant *Nothelaena vellea* and studying its protein profile during drought stress against a non resurrection plant *Nephrolepsis sp. Iranian J. Biol*., **15**: 43-48.

Ghasempour HR, Anderson EM and Gaff Donald F. 2001. Effects of growth substances on the protoplasmic drought tolerance of leaf cells of the resurrection grass, *Sporobolus stapfianus*. *Aust J Plant Physiol.*, **28:** 1115-1120 .

Hassan HM. 2004. Chitinase from the biocontrol agents: *Trichoderma* spp. *Afr. J. Mycol. Biotechnol*., **12**: 45-58.

Holbrook CC, Knauft DA, Dikson DW. 1983 A technique for screening peanut for resistance to *Meloidogyne arenaria*. *Plant Dis*., **57**:957–958

Hurkman WJ, Tanaka CK and Dupont FM. 1988. The Effects of Salt Stress on Polypeptides in Membrane Fractions from Barley Roots, *Plant Physiol*., **88**; 1263–1273.

Ismail AE, Soliman GM and Badr UM. 2018. Genetic improvement of nematotoxicity of *Serratia liquefaciens* against *Meloidogyne incognita* infected eggplant by gamma irradiation in Egypt. *Pakistan J of Parasitol*., **66 (3):** 28-38.

Kashyap AS, Dinesh S, Amit KK and Ravinder PS. 2020. Characterization of plant growth-promoting rhizobacteria isolated from chilli rhizosphere of southern plateau and hills region. *Int. J. of Current Microbiology and Appl Sci.,* **9** (08): 3473- 3483, https://doi.org/10.20546/ijcmas.908.402

Kashyap AS, Manzar N, Nebapure SM, Rajawat MVS, Deo MM, Singh JP, Kesharwani AK, Singh RP, Dubey SC and Singh D. 2022. Unraveling microbial volatile elicitors using a transparent methodology for induction of systemic resistance and regulation of antioxidant genes at expression levels in chili against bacterial wilt disease. *Antioxidants.,* **11**: 404, https://doi.org/10.3390/antiox11020404.

Kashyap AS, Manzar N, Rajawat MVS, Kesharwani AK, Singh RP, Dubey SC, Pattanayak D, Dhar S, Lal SK and Singh D. 2021. screening and biocontrol potential of rhizobacteria native to gangetic plains and hilly regions to induce systemic resistance and promote plant growth in chilli against bacterial wilt disease. *Plants.,* **10:** 2125, https://doi.org/10.3390/plants10102125

Kassab SA, Eissa MFM, Bader UM, Ismail AE, Abdel Razik, BA, and Soliman, GM. 2017. The nematocidial effect of a wild type of *Serratia marcescens* and their mutants against *Meloidogyne incognita* juveniles. *Egypt J. Agronematol.,* **16 (2):**95-114.

Kayani MZ, Mukhtar T and Hussain MA. 2017. Interaction between nematode inoculum density and plant age on growth and yield of cucumber and reproduction of *Meloidogyne incognita* . *Crop Prot.*, **92**: 207-212.

Kumar K. K and Arthurs S. 2021. Recent advances in the biological control of citrus nematodes: A review. *Biological Control.*, **157**:10459.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*., **227**: 680-685.

Lee Y and Kim K. 2016. Antagonistic potential of *Bacillus pumilus*L1 against root-knot nematode, *Meloidogyne arenaria*. *J Phytopathol.*, **164**: 29-39.

Lian LH, Tian BY, Xiong R, Zhu M Z, ZuJ and Zhang K Q. 2007. Proteases from *Bacillus*: a new insight into the mechanism of action for rhizobacterial suppression of nematode, *Letters in Applied Microbiology.*, **45:**262-269.

Migunova VD and Sasanelli N. 2021. Bacteria as biocontrol tool against phytoparasitic nematodes. *Plants.*, **10**: 389.

Mitiku M. 2018. Plant-parasitic nematodes and their management.. A review. Agric. *Res. Technol*., **8**:30–38.

Mohamed SAH, Ameen HH, Elkelany US, El Wakeel MA, Hammam MMA, Soliman, GM. 2021. Genetic improvement of Pseudomonas aeruginosa and *Bacillus cereus* for controlling root knot nematode and two weeds under laboratory conditions. *Jordan J Biol Sci.*, **14 (4):** 859-865.

Mukhtar T, Hussain MA and Kayani MZ. 2017. Interaction between nematode inoculum density and plant age on growth and yield of cucumber and reproduction of *Meloidogyne incognita*. *Bragantia.*, **75**: 108-112.

Netscher C and Sikora RA. 1990. Nematode parasites of vegetables. Pp. 237-283 in M. Luc, R. A. Sikora, and J. Bridge, eds. Plant parasitic nematodes in subtropical and tropical agriculture, CAB International, Wallingford, Oxon, UK.

Priyank H, Chinnannan K, Kadirvelu K, *et al*. 2018. Plant growth promoting rhizobacteria (PGPR): A potential alternative tool for Nematodes biocontrol. *Biocatal Agr Biotechnol*., **17**: 119-128.

Puntener W. 1981. Manual for field trials in plant protection. Agric. Div Geigy Limited, Basle, Switzerland, 205p.

Rakib K, Nain Z, Ahammed B, Karim, M. 2020. Developing *Pseudomonas aeruginosa* mutants with hyperproteolytic activity through UV mutagenesis and characterization for optimized production. **3**: 135- 142, https://doi.org/10.5455/jabet.2020.d118.

Rey M, Delgado JJ, Rincon AM, Limon CM, Benitez T, Perez EA. 2000. Improvement of *Trichoderma* strains for biocontrol. *Rev., Iberoam Micl*., **17:**531-536

Rika A, Nyoman P and Tati S. 2017.Role of Indigenous Rhizosphere Bacteria in Suppressing Root-knot Nematode and Improve Plant Growth Tomato. *Plant Pathol J*., **16**: 25-32.

Sammour RH. 2014. Cultivars identification based on biochemical markers. *RRBS*., **8 (9)**:347-358.

Mohamed SAH, El-Sayed GM, Elkelany US, Youssef MMA, El-Nagdi WMA, Soliman GM. 2021. A local *Bacillus* spp.: isolation, genetic improvement, nematode biocontrol, and nitrogen fixation. *Egyptian pharmaceutical journal.*, **20 (3**):352-363.

Shafique, T, Shafique J, Zahid S, Kazi M, Alnemer O, Ahmad A. 2021. Screening selection and development of *Bacillus subtilis* apr-IBL04 for hyper production of macromolecule alkaline protease. *Saudi Journal of Biological Sciences.*, **28,** 1494-1501, https://doi.org/10.1016/j.sjbs.2020.11.079.

Sharaf AMA , Kailla AM , Mohamed S. Attia MS and Mohamed M, Nofa MM. 2016. Induced resistance in tomato plants against root knot nematode using biotic and abiotic inducers. *Int. J. Adv. Res. Biol. Sci*., **3(11):** 31-46

Shumi W Hossain T and Anwar MN. 2004. Proteolytic Activity of a Bacterial Isolate *Bacillus. fastidiosus. J.B.S*., **4**: 370-374.

Sidhu H. 2018. Potential of plant growth-promoting Rhizobacteria in the management of nematodes: A review. *J Entomol Zoology Studies.*, **6**: 1536-1545.

Singh NK, Handa AK, Hasegawa PM, and Bressan R. 1985. Proteins Associated with Adaptation of Cultured Tobacco Cells to NaCl, *Plant Physiol*., **79**: 126–137.

Smith H, Gallop R C, Patricia W, Harris-Smith and Stanley JL.1952.Sofy AR, Attia MS, Sharaf AMA and El-Dougdoug KA. 2014. Potential impacts of seed bacterization or salix extract in faba bean for enhancing protection against bean yellow mosaic disease. *Nature and Science*., **12(10):** 67-82.

Soliman GM, Mohamed HAH., Haggag LF, and El-Hady ES. 2020. Efficiency of biological control of root-knot nematodes in infected grapevines seedling by genetic Improved bacteria. *Plant Archives.,* **20 (1)**: 951-961.

Soliman, GM, Ameen, HH, Abdel-Aziz, M. and E-lsayed, GM. 2019. In vitro evaluation of some isolated bacteria against the plant parasite nematode *Meloidogyne incognita. Bull. NRC.,* **43:**171, https://doi.org/10.1186/s42269-019-0200-0

Stegmann H. 1979. Electrophoresis and focusing in slabs using the Pantaphor apparatus for analytical and preparative separations in gel (Polyacrylamide, Agarose, Starch, Sephadex). Messeweg 11, D-3300, *Braunschweig Institute of Biochemistry*, West-Germany., pp: 1-29.

Studier FW. 1973.Analysis of bacteriophage T7 early RNA and protein of slab gels. *Molecular. Biol.,***79**:237-248.

Trudgill DL and Blok VC. 2001. Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. *Annual Review of Phytopathol.,* **39**: 53-77.

Van Loon LC, Pierpoint WS, Boller T and Conejero V. 1994. Recommendations for naming plant pathogenesis related proteins. *Plant Molecular Biology Reporte.*, **12**: 245-264.

Vyomesh SP, Pitambara and ShuklaYM. 2018. Proteomics study during root knot nematode (*Meloidogyne incognita*) infection in tomato (*Solanum lycopersicum* L.). *J of Pharmacognosy and Phytochemistry* **.,** 7 **(3**): 1740-1747

Ramadan WA and. Soliman GM**.** 2020 Effect of different applications of bio-agent *Achromobacter xylosoxidan*s against *Meloidogyne incognita* and gene expression in infected eggplant. *Jordan J of Biol Sci.,* **13(3**): 363–370

Wang H, Liu D, Liu Y, Cheng C, Ma Q, Huang Q, Zhang Y. 2007. Screening and mutagenesis of a novel *Bacillus pumilus* strain producing alkaline protease for dehairing. *Lett Appl Microbiol.*, **44**:1–6. doi:10.1111/j.1472-765X.2006.02039.x

Xiang N Kathy S and Donald A. 2018. Biological control potential of plant growth-promoting rhizobacteria suppression of *Meloidogyne incognita* on cotton and *Heterodera glycines* on soybean: A review. *J Phytopathol.*, **166** :449-458.

Zeck WM. 1971. A rating scheme for field evaluation of root-knot infestations. *Pflanzenschutz-Nachr.,* **24**: 141–144.