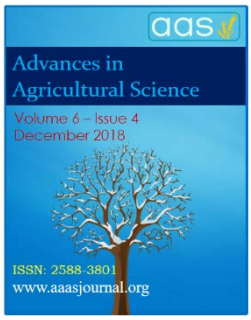
Advances in Agricultural Science

Volume 6 (2018), Issue 04, 52-59

Journal homepage: www.aaasjournal.org

ISSN: 2588-3801



Research Article Open Access

Nematicidal Effects of *Bacillus subtilis* and *Bacillus pumilus* Against *Meloidogyne incognita* infecting Pea

Wafaa M. A. El-Nagdi [1]*, H. Abd-El-Khair [2] and Mona G. Dawood [3]

[1] *Nematology Laboratory, National Research Centre, Giza, Egypt.*

[2] *Plant Pathology Department, National Research Centre, Giza, Egypt.*

[3] *Botany Department , National Research Centre, Giza, Egypt.*

ARTICLE INFO

Article history:
Received: April 13, 2018
Revised: June 19, 2018
Accepted: July 20, 2018
Available online: December 15, 2018

Keywords:
Bacillus subtilis
Bacillus pumilus
Meloidogyne incognita
Pea
Biological control

* Corresponding Author;
E. Mail: wafaaelnagdi@yahoo.com

ABSTRACT

Bacillus subtilis (BS) and *B. pumilus* (BP1 and BP2) were applied alone as well as in combination for controlling *Meloidogyne incognita* infecting pea in pot experiment. Single treatments of bacteria significantly reduced the numbers of juvenile (J_2) in soil (50-80%) ,J_2 in roots (57-78%), females (55-74%), galls (65-74%) and egg-masses (56-70%) , while the combination treatments of them significantly reduced the same parameters in the ranges of 29-76% , 61-77% , 46-69% , 47-67% and 40-61% , compared to 71 , 70 , 70 ,68 and 65% , with Carbofuran 10%, respectively. Results showed that BP2 highly reduced the J_2 in soil , BS highly reduced J_2 in roots BS and BP1 highly reduced the numbers of females, galls and egg-masses in roots. On the other hand, BP1 + BP2 highly reduced the numbers of J_2 in soil, J_2, females and egg-masses in roots, while BS+BP1+BP2 treatment highly reduced the galls number. *B. subtilis* and *B. pumilus*, as single treatment, in some parameters, had more nematicidal activity against *M. incognita*, than in combination treatments. Treatments also significantly increased the growth parameters of pea plants shoot length, shoot fresh and dry weight, leaves numbers and pod fresh and dry weight. The treatments improved the soluble protein and total phenolic compounds in treated pea plants.

Introduction

Meloidogyne incognita Kofoid and White Chitwood (root knot nematode) attacked pea plants (*Pisum sativum* L.) and cause severe damage (Samaliev and Stoyanov2008 and Satyandra *et al*., 2011). Chemical treatments can be managed of root-knot nematodes but application of the nematicides are expensive, non-eco-friendly and causing risk to human and environmental (Abd-Elgawad, 2008). Therefore, biological control is safe and cheap alternative method to chemical controls and its ability to antagonize the nematodes by different mode of action (Gowen and Ahmad,1990 and Choudhary and Johri, 2009).

The colonization of plant roots is useful step for beneficial rhizobacteria act , as biocontrol agents as well as growth-promoting bacteria, depended on microcolonies or biofilms of root exudation (Choudhary and Johri. 2009). Therefore, *Bacillus*

spp. considered one of the important bacterial genera plays an important role in suppressive nematode invasion (Kloepper and Ryu. 2006). *Bacillus* spp. is effective in root colonization, versatile activity against multiple nematodes as well as ability to produce endospores when environmental conditions are stressful and the most species of *Bacillus* are harmless saprophytes (Siddiqui and Mahmood. 1999 and Dawar *et al*., 2008). *B. subtilis* and *B. pumilus* exhibited larvicidal activity against the second stage juveniles (J_2) of *M. incognita in vitro* (Gokte and Swarup. 1988). Application of *Bacillus* spp. significantly reduced hatching of larvae of *Meloidogyne javanica*, whereas mortality of larvae was significantly increased with the increase in time. The growth parameters cowpea and mash bean *viz*. shoot length, root length, shoot weight and root weight significantly increased in treated plants, compared to control (Dawar *et al*., 2008). *B. subtilis* had highest efficacy against *M. arenaria* in potato, where nematode development in treated plant roots was lower than the untreated control (Mohamedova and Samaliev. 2011). Application of the two isolates of *B. pumilus* against *M. javanica* reduced the number of galls and eggs in tomato production. Significant enhancement in root and shoot length and dry root and shoot weight was recorded (Moghaddam *et al*., 2014). In previous study, Abd-El-Khair *et al*. (2016) mentioned that *Bacillus subtilis* and *Bacillus pumilus* showed the highest net mortality of *M. incognita* about ≥ 95% in bioassay test.

The objective of this research was to study the nematicidal activity of *Bacillus subtilis* and *B. pumilus* in pea under pots experiment.

Materials and Methods

Identification, Culturing and Extraction of M. incognita

Adult females were isolated from galled roots of pea plants and identified as *M. incognita* by examination of their cuticular perineal patterns and morphological characteristics according to method described by Taylor and Sasser (1978). The nematode was reared on eggplant in greenhouse at 30 ± 5 ºC. Second stage juveniles (J_2) of *M. incognita* were extracted from these cultures by incubating infected roots in water for three days at 30 ± 5 ºC and then hatched J_2 were collected and counted.

Preparation of Bacillus species inoculums

Cultures of *Bacillus* species *viz*., *B. subtilis* (BS isolate) and *B. pumilus* (BP1 and BP2 isolates) were obtained from Department of Plant Pathology, National Research Centre, Egypt. For the preparation of *Bacillus* species inoculums, pure culture tubes of BS, BP1 and BP2 were incubated for 48 h at (30±2)°C for the multiplication of bacteria. For mass production, one-liter Conical flasks containing 500 ml of broth nutrient glucose (2%) medium (3 g beef extract; 5 g peptone, 20 g glucose, in 1000 ml distilled water and pH at 7.2) were autoclaved .Then, each flask was separately inoculated with 1.0 ml of tested bacteria suspension. The flasks were kept at (30±2) °C in for 48 h and were shaken two times a day. Inoculums culture of bacteria was mixed and adjusted to 10^7-10^9 colony forming unit (CFU)/ml using dilution method. as previously mentioned.

Pots experiment

The experiment was conducted to assess the nematicidal effects of BS, BP1 and BP2 against *M. incognita* under glasshouse conditions in a greenhouse of Department of Plant Pathology, NRC. The plastic pots (20 cm diameter), containing 2 kg of a sterilized mixture of sandy and loamy soil (1:1, w/w), were arranged according to a completely randomized design on a bench in the glasshouse. Seeds were sown in pot in April at 2017. After seed germination, each pot was thinned to two plants. Then, the pots were inoculated (in four holes made

by wooden stalk around the plant roots in the soil) with 1000 newly hatched J_2 of *M. incognita.* At the same time of nematode inoculation, *Bacillus* spp. *viz*. BS, BP1 and BP2 was applied, as soil drenching, at 30 ml of bacterial suspension [10^{7-9} colony forming unit (CFU)/ml]. The sterilized soil was separately mixed with bacterial suspension and then the pots watered. Pots without bacterial suspension served as control. These treatments were compared Furadan (Carbofuran 10%) as a nematicide, at the rate of 0.02g/pot (equivalent to 10kg Fed.). Six replicated pots were used per treatment as well as for the control.

After 60 days of growth, the nematicidal effects of tested *Bacillus* species against *M. incognita* parameters *viz*. numbers of J_2 in soil as well as J_2 , galls , females and egg-masses in roots of pea plants (six plant roots per treatment) and the percentages of reduction were determined. Reduction of nematode parameters was calculated according to the following formula:

$$\text{Nematode parameter reduction (\%)} = \frac{\text{Number in control-Number in treatment}}{\text{Number in control}} \times 100$$

The growth parameters of pea plants, *viz*. shoot length, shoot fresh and dry weight, leaves numbers and pod fresh and dry weight were recorded. Increase in growth parameters was calculated according to the following formula:

$$\text{Growth parameter increase (\%)} = \frac{\text{Reading in treatment- Reading in control}}{\text{Reading in control}} \times 100$$

Total protein content in dry pea seeds was determined according to Bradford method (1976), using a bovine serum albumin as a standard. Two grams of each sample were separately grinded in mortar . Then, 5ml of phosphate buffer (pH 7.6) was added and then transformed to the centrifuge tubes. The sample was centrifuged at 8000 rpm for 20 minutes After extraction, 30µl of each sample was separately mixed with 70µl of distilled water in separate tube. Coosmassic Brillaint Blue solution was added .A 3 ml as total volume was made and then the tubes were incubated for 5 minutes at room temperature. The total protein content was measured as the absorbance by spectrophotometer against the control at 600 nm.

Soluble sugar content was determined in the dry seeds using the colorimetric method described by Dubois *et al.* (1956**)**. One dried seeds was weighed and then 10 ml ethanol 70% was added . Then, the mixtures were kept in refrigerator for 1 week. One ml of the supernatant was mixed with 1 ml distilled water. Then, 1 ml of phenol 5% and 5 ml pure sulfuric acid were added to the solution. Soluble sugar was measured as the absorbance by spectrophotometer against the control at 490 nm. To obtain the standard curve of soluble sugars using, different concentrations of glucose were used.

Total phenolic compounds were extracted from dry seeds and determined calorimetrically according to the method defined by Snell and Snell (1953) using Folin Ciocalteu phenol reagent.

One gram of pea seeds was macerated in 5-10 ml ethanol (80%) for at least 24 h at zero °C, the alcohol was clarified.

The remained residue was re-extracted for 3 times with 5-10 ml of ethanol. At the end, the clarified extract was completed to 50 ml using ethanol .

Statistical analysis

Nematode data were normalized before analysis by log transformation. The obtained data were analyzed

using Computer Statistical Package (CO-STATE) User Manual Version 3.03, Barkley Co.USA and means compared with the Duncan's Multiple Range Test at *P*=0.05(Snedecor and Cochran,1980).

Results and Discussion

Application of BS, BP1 and BP2, as single or in combined as soil treatment with *M. incognita* inoculations, significantly reduced the *M. incognita* parameters *viz*. numbers of J_2 in soil , J_2 in roots , females , galls and egg-masses , where the reduction percentages were in the ranges of 60-80% , 57-78% , 55-74% , 65-74% and 56-70% , compared to nematode only (Untreated control). Carbofuran 10% reduced the above nematode parameters to 71, 70, 70, 68 and 65%, respectively. Results showed that BP2 highly reduced the J_2 number in soil, followed by BS and BP1, respectively. BS highly reduced the J_2 number in roots, followed by BP2 and BP1, respectively. BP1 highly reduced the numbers of females, galls and egg-masses, followed by BS and BP2, respectively.. In combination treatments, BS+BP1, BS+BP2, BP1+BP2 and BS+BP1+BP2significantly reduced the above nematode parameters in the ranges of 29-76%, 61-77%, 46-69%, 47-67% and 40-61%. BP1 + BP2 highly reduced the numbers of J_2 in soil and roots, followed by BS+BP1, BS+BP1 +BP2 and BS+BP1, respectively. BP1 + BP2 highly reduced the numbers of females and egg-masses, followed by BS+BP1 +BP2, BS+BP1 and BS+BP1, respectively. BS+BP1+BP2 highly reduced the galls number, followed by BP1+BP2, BS+BP1 and BS+BP2, respectively (Table 1)..

It is cleared that as single treatment , BP2 highly reduced the J_2 in soil , BS highly reduced J_2 in roots BS and BP1 highly reduced females, galls and egg-masses in roots. On the other hand , in combination treatment, BP1 + BP2 highly reduced the tested nematode parameters. Our results are agreement with those recorded by Priest (1999) and Choudhary and Johri (2009) . They mentioned that the mechanisms of biological agents affecting root gall development, egg hatching or nematode survival were either directly through the production of toxic metabolites or indirectly by induction of systematic resistant. Sarangi and Ramakrishnan, (2016) also that *Bacillus* spp. had the nematicidal activity , may be due to the bacteria able to produce a wide variety of secondary metabolites *viz*. lipopeptide antibiotics of Surfactin and iturin A. *Bacillus* species also can kill nematodes by Bacilli that affect nematode development, fecundity and survival (Zheng *et al*., 2016). *B. pumilus* able to produce the protease and chitinase that demonstrated their ability as a potential biocontrol agent against root-knot nematode (Ahmadian *et al*., 2007 and Lee and Kim, 2016). *B. subtilis* and *B. pumilus* had antibiotic activity (Leifert *et al*., 1995) as well as application of *Bacillus* spp. significantly reduced hatching of larvae of root- knot (Dawar *et al*., 2008) . In pot experiment , El-Nagdi and Abd-El-Khair (2017) reported that *B.subtilis* and *B. megaterium* had nematicidal activity against *M. incognita* in Cowpea . In field application the same bacterial bio-agents controlled *M. incognita* in bean plants under natural infection conditions (El-Nagdi and Abd-El-Khair, 2014). Application of BS, BP1 and BP2, as single soil treatment together with *M. incognita* inoculations, significantly increased the growth parameters of pea plants *viz.* shoot length , shoot fresh weight , shoot dry weight, leaves numbers, pod fresh weight and pod dry weight in the ranges of 60-102%, 71-129% , 30-60% , 31-39%, 21-86% and 21-43% as well as the growth parameters of 48, 40, 25, 14, 18 and 14% with Carbofuran 10% comparing to the control, respectively. BP2 highly increased the growth parameters *viz*. shoot fresh and dry weight and pod fresh and pod dry weight, followed by BP1 and BS, respectively. BP1 highly increased the shoot length and leaves numbers, followed by BP2 and BS, respectively. In the combined treatments, BS+BP1, BS+BP2, BP1+BP2 and BS+BP1+BP2 significantly increased the above growth parameters in the ranges of 63-86%, 101-172%, 22-70%, 37-43%, 24-96% and 21-50%, respectively. BS + BP1 + BP2 highly increased the shoot fresh weight , leaves numbers and pod fresh

Table 1. Nematicidal activity of *Bacillus subtilis* and *Bacillus pumilus* as single or combined treatments on *Meloidogyne incognita* parameters as log_{10} and reduction (%) in pea plants in pots.

Treatments	log 10 of numbers and reduction (Red.%) of *M. incognita* parameters									
	J_2 no. /200g soil		J_2 no. /5g roots		Females no./5g roots		Galls no. /5g roots		Egg-masses no./5g roots	
	log 10	Red. %	log 10	Red. %	log 10	Red. %	log 10	Red. %	log 10	Red. %
Bacillus subtilis (BS)	2.28bc	77	2.60c	88	1.08cd	64	1.21bcd	65	1.05c	64
Bacillus pumilus (BP1)	2.53b	60	2.89b	57	0.95d	74	1.07d	74	0.89d	74
Bacillus pumilus (BP2)	2.24c	80	2.70c	72	1.09c	55	1.24bc	65	1.14c	55
BS + BP1	2.52b	60	2.62c	77	1.10c	56	1.24bc	63	1.14c	56
BS + BP2	2.78a	29	2.85b	61	1.27b	40	1.32b	46	1.27b	40
BP1+ BP2	2.31bc	76	2.25d	90	1.09c	61	1.12cd	65	1.07c	61
BS + BP1+ BP2	2.46bc	49	2.68c	73	1.02cd	58	1.19bcd	69	1.11c	58
Carbofuran 10%	2.39bc	71	2.25d	90	1.01cd	64	1.10cd	70	1.04c	65
Nematode only	2.93a	-	3.26a	-	1.54a	-	1.59a	-	1.29a	-

Means are averages of six replicates. Means followed by different letter(s)are significantly different according to Duncan's Multiple Range Test at $p \leq 0.05$.

Table 2. Effects of *Bacillus subtilis* and *Bacillus pumilus* as single or combined treatments on growth characteristics in pea plants infected by the root- knot nematode, *Meloidogyne incognita*.

Treatments	Growth parameters								Pod parameters			
	Shoot						Leaves		Fresh weight		Dry weight	
	Length		Fresh weight		Dry weight							
	cm.	Increase %	g	Increase %	g	Increase %	No.	Increase %	g	Increase %	g	Increase %
Bacillus subtilis (BS)	27.67bc	60	2.50bcd	71	1.31f	30	17.83ab	35	1.02g	21	0.17de	21
Bacillus pumilus (BP1)	35.00a	102	2.79abc	91	1.35e	34	19.50ab	39	1.45b	73	0.20ab	43
Bacillus pumilus (BP2)	32.33ab	87	3.34ab	129	1.62b	60	18.33ab	31	1.56a	86	0.20ab	43
BS + BP1	28.17bc	63	2.94abc	101	1.51c	50	19.33ab	38	1.22c	45	0.18cd	29
BS + BP2	32.17ab	86	3.40ab	133	1.23h	22	19.17ab	37	1.04f	24	0.19bc	36
BP1 + BP2	31.67ab	83	3.65ab	150	1.72a	70	19.33ab	38	1.13d	35	0.17de	21
BS + BP1+ BP2	30.67abc	77	3.97a	172	1. 41d	40	20.00a	43	1.05e	96	0.21a	50
Carbofuran 10%	25.82c	48	2.05cd	40	1.26g	25	16.00bc	14	0.99h	18	0.16e	14
Nematode only	17.33d	-	1.07d	-	1.01i	-	14.00c	-	0.74i	-	0.14f	-

Means are averages of six replicates. Means followed by different letter(s)are significantly different according to Duncan's Multiple Range Test at $p \leq 0.05$.

Table 3. Effect of *Bacillus subtilis* and *Bacillus pumilus* on biochemical compounds of pea plants infected by the root- knot nematode, *Meloidogyne incognita*.

Treatments	Biochemical compounds (mg/g)		
	Soluble protein	Soluble sugar	Total phenolic compound
Bacillus subtilis (BS)	10.45a	65.53c	26.21a
Bacillus pumilus (BP1)	8.45cde	61.68de	23.31b
Bacillus pumilus (BP2)	8.73b	76.76a	21.65c
BS + BP1	8.65cd	73.50b	20.35d
BS + BP2	8.33de	51.49f	17.44ef
BP1 + BP2	9.05b	60.35e	18.44e
BS + BP1+ BP2	8.30e	48.80f	17.28ef
Carbofuran 10%	8.14ef	51.75f	17.62ef
Nematode only	7.95f	64.64cd	16.54f

Means are averages of six replicates. Means followed by different letter(s)are significantly different according to Duncan's Multiple Range Test at $p \leq 0.05$.

and dry weight averages, while BS + BP2 and BP1 + BP2 Highly increased shoot length and shoot dry weight, respectively (Table 2). A group of rhizosphere bacteria (rhizobacteria) play an important role a beneficial effect on plant growth such as *Bacillus* spp. by several mechanisms of growth promotion include production of growth stimulating phytohormones, solubilization and mobilization of phosphate, siderophore production, antibiotics production, ethylene synthesis inhibition and induction of plant systemic resistance to pathogens (Richardson *et al*., 2009). Significant enhancement in root and shoot length and dry root and shoot weight in potato plants was recorded by Moghaddam *et al*. (2014).

Effects of *Bacillus* spp. *viz*. BS, BP1 and BP2, as single soil treatment together with *M. incognita* inoculations, on soluble protein, soluble sugar and total phenolic compounds as mg/g in pea seeds are listed in Table (3). The above biochemical compounds ranged from 8.45-10.45 mg/g; 61.68-76.76 mg/g and 21.65-26.21 mg/g when BS, BP1 and BP2 was applied alone, respectively. BS significantly increased the soluble protein and total phenolic compounds, while BP2 highly increased the soluble sugar, respectively. The same compounds ranged from 8.30-9.05 mg/g: 48.80-73.50 mg/g and 16.85-20.35 mg/g in combined treatments of BS+BP1, BS+BP2, BP1+BP2 and BS+BP1+BP2, respectively. BS + BP1 highly increased the soluble protein and total phenolic compounds, while BP1+BP2 highly increased the soluble sugar, respectively. The biochemical compounds were 8.14, 51.75 and 16.64 mg/g with Carbofuran 10% the , compared to 7.95 , 64.64 and 16.64 mg/g in nematode only (Table 3). These results are agreement with those recorded by Shama *et al*. (2010). They indicated that the biocontrol agents viz. *Trichoderma harzianum*, *Pseudomonas fluorescens* and *B. subtilis* enhanced the contents of phenol, soluble sugars, and total protein *Brassica juncea* . The sugar beet plant residues also increased the soluble carbohydrates, total carbohydrates, phenols and soluble proteins in cowpea seeds (Youssef *el al*., 2018).

References

Abd-Elgawad, M.M.M., 2008. The current status of phytonematode management in Egypt with special

reference to applicable nematicides. Egyptian Journal of Agro nematology 6, 33-46.

Abd-El-Khair, H, El-Nagdi, Wafaa, M.A. and Ameen, Hoda, H., 2016. Antagonistic effects of rhizobacteria isolates against *Meloidogyne incognita* infecting tomato plants under greenhouse conditions. International Journal of PharmTech Research 9(10), 097-107.

Ahmadian, G, Degrassi, G, Venturi, V, Zeigler, D R, Soudi, M and Zanguinejad, P., 2007. *Bacillus pumilus* SG2 isolated from saline conditions produces and secretes two chitinases. Journal of Applied of Microbiology 103(4), 1081–1089.

Bradford, MM., 1976. A rapid and sensitive method for quantization of microgram quantities of protein utilizing the principle of protein-dye-binding. Analytical Biochemistry 72, 248-254.

Choudhary, D C and Johri, B N., 2009. Interactions of *Bacillus* spp. and plants – with special reference to induced systemic resistance (ISR). Microbiological Research, 164(5), 493-513.

Dawar, S, Tariq, M and Zaki, M J., 2008. Application of *Bacillus* species in control of *Meloidogyne javanica* (Treub) Chitwood on cowpea and mash bean. Pakistan Journal of Botany, 40(1), 439-444.

Dubois, M, Cilles KA, Hamilton J, Rebers R, Smith F., 1956. Colorimetric method of determination of sugars and related substances. Analytical Chemistry 28, 350-356.

El-Nagdi, Wafaa MA and Abd-El-Khair, H., 2017. Application of certain bacterial and fungal species for controlling *Meloidogyne incognita* parameters in cowpea. International Journal of Entomology and Nematology 3 (2), 70-76.

El-Nagdi, Wafaa MA and Abd-El-Khair, H., 2014. Biological control of *Meloidogyne incognita* and *Fusarium solani* in dry common bean in the field. Archives of Phytopathology and Plant Protection 47(4), 388-397.

Gokte, N. and Swarup, G., 1988. On the potential of some bacterial against root-knot and cyst nematodes. Indian Journal of Nematology 18,152-153.

Gowen, SR. and Ahmad, R., 1990. *Pasteuria penetrans* for control of pathogenic nematodes. Aspects of Applied Biology 24, 25-32.

Hussey, RS and Berker, KR., 1973. A comparison of methods of inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 57, 1025-1028.

Kloepper, J. and Ryu, C., 2006. Bacterial endophytes as elicitors of induced systemic resistance. In; Microbial root endophytes, Eds. *In*; Schulz, B.; Boyle, C. and Siebern, T., Springer-Verlag, Heildelberg, 33-51.

Lee, Y S. and Kim, K Y., 2016. Antagonistic potential of *Bacillus pumilus* L1 against root-knot nematode, *Meloidogyne arenaria***.** Journal of Phytopathology 164 (1), 29–39.

Leifert, C, Li, H, Chidburee, S, Hampson, S,Workman, S, Sigee, D, Epton, HA and Harbour, A. ,1995. Antibiotic production and biocontrol activity by *Bacillus subtilis* CL27 and *Bacillus pumilus* CL45. Journal of Applied Bacteriology 78(2), 97-108.

Moghaddam, M R, Moghaddam, EM, Ravari, S B and Rouhani, H., 2014. The first report of *Bacillus pumilus* influence against *Meloidogyne javanica* in Iran. Journal of Crop Protection 4, 3 (1), 105-112.

Mohamedova, M. and Samaliev, H., 2011. Effect of the rhizobacterium *Bacillus subtilis* on the development of the root-knot nematode *Meloidogyne arenaria* at different temperatures. Agricultural Science and Technology 3 (4), 378 – 383.

Priest, F., 1993. Systematic and ecology of *Bacillus*. *In*: Sonenshein , A.L.; Hoch, J.A. and Losick, R. *Bacillus subtilis* and Other Gram-Positive Bacteria. 2nd ed. American Society for Microbiology Press.

Richardson, AE, Barea JM, McNeill AM, Prigent-Combaret C., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil ,321, 305–339.

Samaliev, H. and Stoyanov, D., 2008. Parasitic nematodes of crop plants and their control. Agriculture University Academic Press, Plovdiv 78-122.

Sarangi, T. and Ramakrishnan, S., 2016. Influence of Biomolecules of *Bacillus* spp. against Phytopathogens: A Review International. Journal of Current Microbiology and Applied Sciences 5 (7),131-134.

Satyandra, S., Bhagawati, B. and Goswami, B.K., 2011. Bio-management of root-knot disease of chick pea caused by *Meloidogyne incognita*. Indian Council of Agricultural Research 19(1), 159-163.

Sharma, A., Haseeb, A and Abuzar, S., 2006. Screening of field pea (*Pisum sativum*) selections for their reactions to root-knot nematode (*Meloidogyne incognita*). Journal of Zhejiang Univristy Science B. 7(3), 209–214.

Sharma , S., Singh, J., Munshi, GD. and Munshi, SK., 2010. Biochemical changes associated with application of biocontrol agents on Indian mustard leaves from plants infected with *Alternaria* blight. Archives of Phytopathology and Plant Protection 43 (4), 315-323.

Siddiqui, IA. and Mahmood, I., 1999. Role of bacteria in the management of plant parasitic nematodes: a review. Bioresource technology 69, 167–179.

Snedecor, GW. and Cochran, WG., 1980. Statistical Methods. 5th ed. Ames, IA: Iowa State Univ. Press; p. 593.

Snell, FD., Snell, CT., 1953. Colorimetric method. Vol. III, Van Nostrand Company, London, p.606.

Taylor, A L. and Sasser, J N., 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). IMP. Raleigh, N.C.: North Carolina State University Graphics; p. 111.

Youssef, MMA., El-Nagdi, Wafaa MA and Dawood, Mona G., 2018. Effect of sugar beet plant residues on population density of root knot nematode, *Meloidogyne incognita* infecting cowpea and biochemical changes in treated plants. Pakistan Journal of Nematology 36 (1), 41-48.

Zheng, Z., Zheng J., Zhang Z., Peng D. and Sun M., 2016. Nematicidal spore-forming Bacilli share similar virulence factors and mechanisms .Scientific Reports, 6 (31341); Doi: 10.1038/srep31341