

Bio-Efficacy of *Bacillus* Species in the Management of Root-Knot Nematode Pest of Pepper

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ABSTRACT

Background and Objective: The *Bacillus* species are proven biological control agents, present in nature, though can be cultured. In this study the *Bacillus* species were evaluated on the field for the management of root knot nematode, *Meloidogyne incognita*, a pest of pepper. **Materials and Methods:** The field experiment was a 2×7 factorial, fitted into randomized complete block design. There were two pepper varieties, seven treatments replicated four times and inoculated with 5,000 juveniles of *M. incognita* at two weeks after transplanting. Data were collected on nematodes and on the growth and yield of pepper. Data were analyzed using analysis of variance and means were compared using Fisher's Least Significant Difference at 5% probability level. **Results:** The tallest pepper (53.00 cm) was from pepper treated with mixture of *B. cereus* and *B. thuringiensis* while the shortest (16.33 cm) was from the control plot. The highest fruit yield (9.0 g) was produced by pepper treated with *B. thuringiensis* while the control had the lowest (1.67 g). Nematode populations were significantly reduced in *Bacillus* treated soil (146.17 in 250 mL soil) compared to control (947.38 in 250 mL soil). **Conclusion:** This study concluded that the application of *Bacillus* species reduced soil nematode population significantly and increased fruit yield of pepper.

KEYWORDS

Capsicum annum, *C. frutescence*, *Bacillus cereus*, *B. subtilis*, *B. thuringiensis*, *Meloidogyne incognita*

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INTRODUCTION

Pepper, a good source of vitamins and antioxidants, is cultivated for vegetable, spice and value-added processed products¹. Fresh fruits of pepper can be processed into paste and bottled for sale. The crop is used as a catch/trap crop to reduce field infestation of the parasite². Bird, cayenne and skin hot peppers are the commonly cultivated in Nigeria. Their fruits which can be used fresh or dried vary in size, color, shape and pungency.

One of the economic important pests of pepper is the plant-parasitic nematodes. Nematodes cause yield loss and quality reduction of pepper which indirectly reduces its economic value³. For instance, root-knot nematode, *Meloidogyne incognita*, has been reported as the most widely and commonly encountered



species of nematode on pepper⁴. They are responsible for about 70-90% yield losses in pepper. In economic terms, losses of about \$157 billion annually have been estimated. Plant parasitic nematodes reduce the functional metabolism and growth of pepper; damage and weakened pepper root and accelerate root decay; cause galls (warty appearances) on the root of pepper. To overcome this effect, management of plant parasitic nematodes becomes imperative so that higher yield and quality of pepper is achieved⁵.

Nematode management is more difficult than other agricultural pests because they inhabit the soil and usually attack the underground part of the plant. Chemical methods have been customarily used to control nematodes. Chemical agents are easy to apply, have rapid effects, but are not eco-friendly and pose serious risk to the environment⁶. Biological control is one of the effective management strategies with little or no hazard. This biological approach predominantly utilizes the microorganism groups, like the fungi and bacteria that are present in the soil biota⁷. There are many nematophagous bacteria that prey on nematode and also produce certain toxins, antibiotics and enzymes. These nematophagous bacteria interfere with nematode-plant-host relationship and induce systemic resistance on plants and promote plant health^{8,9}. These bacteria can form a complex interaction among bacteria, nematodes, plants and the environment to control populations of plant-parasitic nematodes in natural conditions¹⁰. Scientific interest has been stimulated in recent years, in the development of safer and more potent non-chemical pesticides for the management of plant-parasitic nematodes.

This research falls within the economic pillars under the good health and well-being of the vision 2030 Sustainable Development Goals. The research provides foundational background information on the production of safe to eat foods for wellness and healthy life. Therefore, the field experiment was conducted to assess the performances of *Bacillus cereus*, *Bacillus subtilis* and *Bacillus thuringiensis*, singly and in combination on the management of root knot nematode disease of pepper.

MATERIALS AND METHODS

Study area: The research was conducted at the Teaching and Research Farm, Ladoké Akintola University of Technology, Ogbomoso, Nigeria on Latitude and Longitude coordinates of 8.142165°N and 4.245186°E during 2023 cropping season.

Source of pepper seeds: The two varieties of pepper seeds, *Capsicum annum* cv. Y/GBN/VI and long cayenne *Capsicum frutescens* cv. NHVIA, root knot-nematode susceptible pepper varieties, were obtained from National Institute of Horticultural Research (NIHORT), Ibadan, Oyo State, Nigeria.

Source of *Bacillus* species: The three *Bacillus* species (*B. cereus*, *B. subtilis* and *B. thuringiensis*) pure inoculum were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria and culture at the Department of Crop and Environmental Protection, Faculty of Agricultural Sciences, Ladoké Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, on 1.8 g of yeast agar and 10 g of sugar into 1000 mL of distilled water inside a 2000 mL capacity flask, under sterile condition for 3 weeks.

Nursery preparations: A well-drained and hot steam (80-100°C) soil mixture consisting of compost and topsoil in equal proportions (50:50) was used in making seed beds of 1 m long and 0.3 m breadth with a spacing of 1 m in between the beds. Pepper seeds were broadcasted on the beds. Shade was provided with palm fronds while watering was done in morning and evening for 4 weeks before transplanting to the experimental site.

Experimental site and field layout: The experiment was conducted at the Ladoké Akintola University of Technology (LAUTECH), Teaching and Research Farm, Ogbomoso, Nigeria. The selected area was cleared and divided into 4 blocks with 4 replicates for each of the varieties of pepper (*Capsicum annum* cv.

OY/GBN/VI and *Capsicum frutescens* cv. NHVIA). Each block was composed of 7 treatments which were replicated four times giving a total of 56 plots for each variety of pepper and was laid out in 2×7 factorial, fitted into Randomized Complete Block Design (RCBD). Each plot is composed of 2 ridges, each ridge measuring 4 m long and 0.5 m wide.

Routine soil analysis: Soil sample (5 kg) collected with spade at a depth of 0-30 cm from the experimental site was air-dried, sieved through a 5 mm sieve and sub-samples were further sieved through 2.0 and 0.5 mm sieves and then taken to the laboratory for chemical and particle size distribution analyses. Soil pH was determined with the glass-electrode pH meter (Apera type, made in Columbus, Ohio, USA) on 1:1 soil-solution mixture. The organic carbon was determined by Walkey-Black method¹¹ and the total N was by regular macro-Kjeldahl (Manufactured by Velp Scientifica, Usmate, Italy) method. Available P was extracted by the Mehlich¹² method, while the exchangeable cations were extracted with 1N NH₄OAc solution. Calcium, Na and K were measured with the flame photometer and Mg was found using the atomic absorption spectro-photometer (Manufactured by Infitek Co. Ltd, China)¹³. Exchangeable acidity (H⁺) of the soil was determined using titration method. Effective Cation Exchange Capacity (ECEC) was established as the sum of the exchangeable cations, K, Na, Ca and Mg and H⁺ expressed in c mol/kg of soil.

Planting operation: The pepper seedlings were transplanted to the experimental field at 4 weeks after sowing. The seedlings were planted at one seedling per stand and weeding was done at when due.

Extraction and inoculation of nematode: A standard method of Whitehead and Hemming¹⁴ was used to extract the root-knot nematode, *Meloidogyne incognita*, from rootstock of infected *Celosia argentea*. Pepper seedlings were inoculated with *M. incognita*, at two weeks after transplanting, with each plant being inoculated with 5000 juveniles as soil drench within the plant rhizosphere.

Treatment application: The seven treatments used were individual and combination of each of the *Bacillus* species (*B. cereus*, *B. subtilis*, *Bacillus thuringiensis*, *B. cereus*+*B. subtilis*, *B. cereus*+*B. thuringiensis*, *B. subtilis*+*B. thuringiensis* and control). Application rates of 1.0 L of different *Bacillus* species at 1×10⁷ spore per 10 mL into 10 L of water was applied as a soil drench in the 4th week after transplanting.

Determination of nematode population in soil at transplanting and harvest periods: Soil samples were randomly collected from the plots during the period of transplanting of the pepper seedlings and after harvest for nematode extraction. A total of 28 soil samples were taken to the laboratory for nematode extraction using the modified Baermann's technique¹⁵. Nematodes were extracted from 250 cm³ soil, identified and counted using stereomicroscope (manufactured by Infitek, China).

Statistical analysis: Data collected on plant height, number of leaves per plant, number of branches per plant, number of fruits per plant, fruit weight, initial and final nematode population were subjected to Analysis of Variance (ANOVA) and means were separated using the Fisher's Least Significant Difference (LSD) at 5% probability level.

RESULTS

Routine soil analysis of the experimental site: The results of the soil physical properties of the experimental site revealed that the soil texture of the site was sandy with 0.7% clay, 3.3% silt, 31.0% fine sand and 65% coarse sand and chemical analysis had a pH of 6.8 with 9.5% organic carbon, 16.4% organic matter with relatively low soil mineral contents as presented in Table 1.

Table 1: Physical and chemical properties of the soil of the experimental site

Properties	Value
Mechanical properties	
Coarse sand (%)	65.00
Fine sand (%)	31.00
Silt (%)	3.30
Clay (%)	0.70
Chemical analysis	
pH	6.80
Organic carbon (%)	9.50
Organic matter (%)	16.40
Nitrogen (%)	0.57
Calcium (mol/kg)	0.66
Potassium (mg/kg)	0.57
Sodium (mol/kg)	0.54
Micronutrients (ppm)	
Iron (mg/kg)	0.30
Magnesium (mg/kg)	1.05
Zinc (mg/kg)	1.18
Copper (mg/kg)	0.25
Lead (mg/kg)	0.10
Cadmium (mg/kg)	0.12
Chromium (mg/kg)	0.05
Nickel (mg/kg)	0.05

Effects of *Bacillus* spp., on the plant height: The effect of *Bacillus* spp. and pepper variety on plant height of pepper as presented in Table 2 was significantly influenced ($p \leq 0.05$) by the *Bacillus* species between 4 and 8 weeks after transplanting (WAT). Throughout the study, plants treated with *Bacillus* spp. and their combinations resulted in significantly taller plants than control. At 4 and 6 WAT, *B. subtilis* produced the tallest *C. annuum* plants (40.00 and 42.33 cm) respectively, while the combination of *B. cereus* and *B. thuringiensis* resulted in the tallest *C. annuum* plants (45.67 and 47.00 cm), respectively. The tallest *C. annuum* plants at 8 WAT (44.67 cm) was recorded on plots treated with *B. subtilis* while the tallest *C. frutescens* plants (49.00 cm) was recorded on plots treated with the combination of *B. cereus* and *B. thuringiensis*.

Effects of *Bacillus* species on the number of leaves: The effects of *Bacillus* spp. and pepper variety on the number of leaves of pepper were presented in Table 3. The *Bacillus* treatments significantly ($p \leq 0.05$) influenced the number of leaves at 6 and 8 WAT. The pepper varieties significantly ($p \leq 0.05$) determined the number of leaves at 4, 6 and 10 WAT. The *C. frutescens*, plants treated with the combination of *B. cereus* and *B. subtilis* had the highest number of leaves (228) while at 6 WAT, *B. subtilis* produced the highest number of leaves (267.67) for *C. annuum* as well as for *C. frutescens* (266.67). Control plants had the lowest number of leaves. At 8 WAT, the combination of *B. cereus* and *B. thuringiensis* produced the highest number of leaves (297.6) for *C. annuum* while the combination of *B. subtilis* and *B. thuringiensis* produced the highest number of leaves for *C. frutescens* (255). The control plot had the lowest number of leaf per plant.

Effects of *Bacillus* species on the number of branches: The number of branches of pepper as influenced by *Bacillus* spp. and pepper varieties was presented in Table 4. The *Bacillus* treatments significantly ($p \leq 0.05$) influenced the number of branches throughout data collection except at 4 WAT ($p \geq 0.05$) while the pepper variety had no significant effect on the number of branches. At 4 WAT, the highest number of *C. annuum* branches (5.33) was recorded on plants treated with *B. subtilis* though this was not significantly different from the number of branches recorded from other *Bacillus* spp. The highest number of *C. frutescens* branches (4.33) was recorded on plants that were treated with a combination of *B. cereus* and *B. thuringiensis*; for the rest of the observation period, plants treated with *B. thuringiensis*

Table 2: Effects of *Bacillus* species on the plant height (cm) of pepper inoculated with plant parasitic nematode in Ogbomoso

Treatment	4 WAT			6 WAT			8 WAT			10 WAT			12 WAT		
	Bell pepper	Cayenne pepper		Bell pepper	Cayenne pepper		Bell pepper	Cayenne pepper		Bell pepper	Cayenne pepper		Bell pepper	Cayenne pepper	
<i>Bacillus cereus</i>	37.00	40.47		39.67	42.33		41.67	44.67		43.67	46.33		45.00	48.00	
<i>Bacillus cereus</i> + <i>Bacillus subtilis</i>	32.00	37.33		34.00	39.67		36.00	41.33		38.33	43.67		40.33	45.33	
<i>Bacillus cereus</i> + <i>Bacillus thuringiensis</i>	36.67	45.67		38.67	47.00		40.00	49.00		42.00	51.67		44.67	53.00	
<i>Bacillus subtilis</i>	40.00	42.33		42.33	44.67		44.67	46.00		46.33	48.67		48.33	50.00	
<i>Bacillus subtilis</i> + <i>Bacillus thuringiensis</i>	33.33	37.33		35.33	40.00		37.33	42.00		39.33	43.33		41.33	45.67	
<i>Bacillus thuringiensis</i>	29.00	33.67		31.67	35.67		33.00	37.33		35.67	39.00		37.00	40.00	
Control	16.33	17.00		18.67	19.33		21.00	22.67		24.33	25.00		25.00	27.67	
LSD treatment	22.5			13.33			15.33			Ns			Ns		
LSD variety	Ns			Ns			Ns			Ns			Ns		
LSD V×T	Ns			Ns			*			Ns			Ns		

WAT: Weeks after transplanting, *Data significant at $p \leq 0.05$ and Ns: Data not significant at $p > 0.05$

Table 3: Effects of *Bacillus* species on the number of leaves of pepper inoculated with plant parasitic nematodes in Ogbomoso

Treatment	4 WAT			6 WAT			8 WAT			10 WAT			12 WAT		
	Bell pepper	Cayenne pepper		Bell pepper	Cayenne pepper		Bell pepper	Cayenne pepper		Bell pepper	Cayenne pepper		Bell pepper	Cayenne pepper	
<i>Bacillus cereus</i>	210.67	122.00		239.33	144.00		176.00	102.00		227.33	133.33		265.67	20.33	
<i>Bacillus cereus</i> + <i>Bacillus subtilis</i>	256.67	228.00		190.67	112.67		181.00	102.33		180.00	120.67		223.00	120.67	
<i>Bacillus cereus</i> + <i>Bacillus thuringiensis</i>	191.33	194.67		144.67	121.33		294.67	220.00		127.33	121.33		155.33	174.00	
<i>Bacillus subtilis</i>	260.67	214.67		268.67	266.67		174.33	172.67		170.67	178.33		164.67	176.67	
<i>Bacillus subtilis</i> + <i>Bacillus thuringiensis</i>	188.00	156.67		244.67	120.67		184.00	255.00		268.00	88.67		148.67	236.00	
<i>Bacillus thuringiensis</i>	264.67	114.00		248.67	72.67		290.67	111.00		155.33	104.00		126.67	107.33	
Control	312.00	88.00		111.33	80.67		79.33	82.00		52.00	85.33		35.33	85.33	
LSD treatment	Ns			171.67			176.66			Ns			Ns		
LSD variety	80.76			75.63			Ns			49.86			Ns		
LSD V×T	Ns			Ns			Ns			Ns			*		

WAT: Weeks after transplanting, *Data significant at $p \leq 0.05$ and Ns: Data not significant at $p > 0.05$

Table 4: Effects of *Bacillus* species on the number of branches of pepper inoculated with plant parasitic nematodes in Ogbomoso

Treatment	4 WAT			6 WAT			8 WAT			10 WAT			12 WAT		
	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper	
<i>Bacillus cereus</i>	5.00	3.00	5.33	6.00	6.33	6.67	9.00	7.33	9.33	7.33	9.33	7.33	9.33	7.33	
<i>Bacillus cereus</i> + <i>Bacillus subtilis</i>	3.33	3.33	4.67	4.67	5.33	5.67	8.33	8.33	9.00	8.33	8.33	8.33	9.00	8.33	
<i>Bacillus cereus</i> + <i>Bacillus thuringiensis</i>	3.00	4.33	4.00	6.33	6.33	7.33	6.67	7.67	7.67	6.67	7.67	8.67	7.67	8.67	
<i>Bacillus subtilis</i>	5.33	3.67	8.33	5.33	8.67	8.00	10.00	8.00	12.67	8.00	8.00	12.67	8.67	8.67	
<i>Bacillus subtilis</i> + <i>Bacillus thuringiensis</i>	3.33	4.00	6.33	5.67	8.00	6.67	8.67	8.00	6.00	8.00	8.00	8.67	6.00	8.67	
<i>Bacillus thuringiensis</i>	5.00	2.00	10.33	7.00	10.67	8.33	12.00	8.33	15.33	8.33	8.33	15.33	8.33	8.33	
Control	2.33	3.00	3.33	4.00	4.33	5.00	4.33	5.00	5.00	5.00	5.00	5.00	5.00	5.00	
LSD treatment	Ns		2.67		2.17		2.34		5.83		5.83		5.83		
LSD variety	Ns		Ns		Ns		Ns		Ns		Ns		Ns		
LSD V×T	**		**		*		**		**		**		**		

WAT: Weeks after transplanting, **Data significant at p<0.05 and Ns: Data not significant at p>0.05

Table 5: Effects of *Bacillus* species on the number of flowers of pepper inoculated with plant parasitic nematodes in Ogbomoso

Treatment	4 WAT			6 WAT			8 WAT			10 WAT			12 WAT		
	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper	
<i>Bacillus cereus</i>	19.33	11.67	22.33	9.67	21.67	15.33	16.00	10.00	13.67	10.00	13.67	4.33	13.67	4.33	
<i>Bacillus cereus</i> + <i>Bacillus subtilis</i>	26.67	22.00	16.67	22.67	11.33	18.33	10.00	15.00	10.67	15.00	10.67	31.00	10.67	31.00	
<i>Bacillus cereus</i> + <i>Bacillus thuringiensis</i>	29.00	23.00	21.67	20.67	13.00	18.00	26.00	18.67	12.00	18.67	12.00	11.33	12.00	11.33	
<i>Bacillus subtilis</i>	36.67	34.33	15.00	13.33	19.33	17.00	16.00	15.33	20.67	15.33	20.67	24.67	20.67	24.67	
<i>Bacillus subtilis</i> + <i>Bacillus thuringiensis</i>	19.33	24.67	34.00	18.00	25.67	19.33	25.67	15.00	12.00	15.00	12.00	24.00	12.00	24.00	
<i>Bacillus thuringiensis</i>	23.67	10.00	15.33	14.67	14.00	21.67	9.00	15.00	12.00	15.00	12.00	15.00	12.00	15.00	
Control	12.33	10.00	9.67	8.00	8.67	8.00	12.00	9.67	3.33	9.67	3.33	10.33	3.33	10.33	
LSD treatment	19.67		17.70		14.17		Ns		Ns		Ns		Ns		
LSD variety	Ns		Ns		Ns		Ns		Ns		Ns		Ns		
LSD V×T	Ns		Ns		*		Ns		Ns		Ns		Ns		

WAT: Weeks after transplanting, *Data significant at p<0.05 and Ns: Data not significant at p>0.05

Table 6: Effects of *Bacillus* species on yield of pepper inoculated with parasitic nematode in Ogbomoso

Treatment	Number of fruits		Weight of fruit (g)	
	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper
<i>Bacillus cereus</i>	2.33	1.67	14.33	22.00
<i>Bacillus cereus</i> + <i>Bacillus subtilis</i>	2.00	1.67	22.67	32.00
<i>Bacillus cereus</i> + <i>Bacillus thuringiensis</i>	3.33	2.00	17.00	35.00
<i>Bacillus subtilis</i>	6.33	2.33	21.00	24.67
<i>Bacillus subtilis</i> + <i>Bacillus thuringiensis</i>	2.00	1.67	15.33	25.00
<i>Bacillus thuringiensis</i>	9.00	6.00	49.00	71.33
Control	8.00	1.67	3.67	19.67
LSD treatment	4.83		32.84	
LSD variety	2.28		Ns	
LSD V×T	*		Ns	

*Data significant at $p \leq 0.05$ and Ns: Data not significant at $p > 0.05$

Table 7: Effects of *Bacillus* species on nematode infestation of pepper in Ogbomoso

Treatment	Initial nematode population		Final nematode population	
	Bell pepper	Cayenne pepper	Bell pepper	Cayenne pepper
<i>Bacillus cereus</i>	209.00	210.00	165.00	176.00
<i>Bacillus cereus</i> + <i>Bacillus subtilis</i>	210.00	209.67	140.67	151.67
<i>Bacillus cereus</i> + <i>Bacillus thuringiensis</i>	209.33	210.00	230.00	230.00
<i>Bacillus subtilis</i>	209.00	210.33	150.00	171.67
<i>Bacillus subtilis</i> + <i>Bacillus thuringiensis</i>	209.67	208.00	215.00	213.33
<i>Bacillus thuringiensis</i>	209.33	211.00	243.33	238.33
Control	210.00	210.00	936.67	959.00
LSD treatment	Ns		707.00	
LSD variety	Ns		Ns	
LSD V×T	Ns		Ns	

Ns: Data not significant at $p > 0.05$

consistently had the highest number of branches for the two pepper varieties and this was not significantly different from the number of branches recorded from plants treated with *B. subtilis* and *B. subtilis* plus *B. thuringiensis* combination. Untreated plants (control) steadily produced the lowest number of branches while *C. frutescens* also constantly produced the highest number of branches throughout the experiment. The interaction between the *Bacillus* spp. and pepper variety significant ($p \leq 0.05$) determined the number of branches of pepper.

Effects of *Bacillus* species on the number of flowers: The effect of *Bacillus* spp. and pepper variety on the number of flowers produced by pepper was presented in Table 5. Application of *Bacillus* spp. had significant effects ($p \leq 0.05$) on the number of flowers produced between 4 and 8 WAT. But there was no significant difference between the number of flowers produced at 10 and 12 WAT ($p \geq 0.05$). Pepper variety had no significant effect on the number of flowers. Untreated pepper (control) had the lowest number of flowers.

Capsicum frutescens significantly produced the highest number of flowers except at 8 and 12 WAT. The interaction between the *Bacillus* spp. and pepper variety significantly ($p \leq 0.05$) influenced the number of flowers at 8 WAT.

Effects of *Bacillus* species on the yield: The effects of *Bacillus* spp. and pepper variety on the yield parameters of pepper was presented in Table 6. Both the number and weight of fruits were significantly influenced ($p \leq 0.05$) by the *Bacillus* spp. With regards to the pepper variety, the number of fruits was significantly affected ($p \leq 0.05$) by the variety while the weight of fruits was not significantly affected ($p > 0.05$). Pepper that was treated with *B. thuringiensis* consistently produced the highest number of fruits for *C. annuum* and *C. frutescens* (9.0 and 6.0, respectively) as well as increased weight of fruits for

C. annuum and *C. frutescens* (49.0 and 71.33 g, respectively) while the values for both parameters did not significantly differ among the other *Bacillus* spp. The controls had lowest number of fruit and fruit weight. Interaction between the *Bacillus* spp. and the pepper variety had a significant ($p \leq 0.05$) effect on the number of fruits.

Effects of *Bacillus* species on the nematode population: The effects of *Bacillus* spp. and pepper variety on the nematode infestation of pepper was presented in Table 7. *Bacillus* spp. significantly ($p \leq 0.05$) affected the final nematode population in the soil. Plots treated with a combination of *B. cereus* and *B. subtilis* had the lowest nematode population on plots that contained *C. annuum* (140.67) and *C. frutescens* (151.67).

DISCUSSION

There are various reports on root-knot nematodes, as the most economic important plant parasitic nematodes, because they infest most of the economically important crop species. Reports of successful control of plant-parasitic nematodes with medicinal plant have been given by some researchers¹⁵. One valuable substitute that is gaining popularity in nematode management is biological control, predominantly involving microorganisms like fungi and bacteria⁷.

The *Bacillus* species can effectively control nematode infestation on the pepper plants as observed on the growth parameters compared to plants that received no *Bacillus* treatment (control). This agreed with the findings of some researchers who worked medicinal plants and bio-control agents and reported that bio-control agents improve the growth of crops that are infected with plant-parasitic nematodes. The findings of this report, however, indicates that the different *Bacillus* spp. that were evaluated have different efficiency against the root-knot nematodes. In addition, the non-significant variation observed for most of the growth parameters at 4 WAT may be due to the fact that the inoculated *Bacillus* spp. are yet to be fully activated.

Although chemical agents like carbofuran are efficient in controlling nematodes¹⁶, their persistence may pose ecological problems¹⁷. Therefore, bio-control is suggested as a safer solution. Various fungal antagonists of nematodes have shown promising results. In this study, the *Bacillus* species are promising in the control of the root-knot nematode infestation by significant improved growth and yield of pepper.

Incorporation of *Bacillus* spp. into nematode infested soil sown to pepper reduced the population density of root-knot nematode and increased the fruit yield. Higher fruit yield recorded in plots amended with the *Bacillus* spp., might be due to a reduction in nematode population in the soil, thus resulting in an overall reduction in injuries to the root system and hence a more efficient use of soil nutrients^{18,19}. The *Bacillus* spp., may have killed the juveniles of the root-knot nematodes in the soil through different mechanisms including parasitism of juveniles, adults and eggs as well as through nematode trapping. This can also be due to improved activities of other microorganisms activated by the bio-control bacteria amendments; many of which are reported to be antagonistic to phyto-nematodes.

CONCLUSION

In the present study, application of *Bacillus* species effectively controlled the population of root-knot nematode in pepper fields. However, application of *B. subtilis*, *B. cereus*, *B. thuringiensis* and their combinations were effective bio-control treatment in managing parasitic nematodes.

SIGNIFICANCE STATEMENT

Due to public concern about the risks associated with synthetic pesticides, researchers have geared their efforts to find permanent solution to the problems through the use of bio-control agents. Residues of synthetic pesticides have been found in crop, human, livestock, soil and water. Synthetic pesticide, a

source of carcinogenic diseases and food poisons, has been banned because of their high toxicity and hazardous effect on the crops, environment, soil and consumers. One of the outstanding ways out of these problems is the application of bio-control agents, e.g. *Bacillus*, *Pseudomonas* and *Trichoderma* species. The current research therefore addresses the application of *Bacillus* species in the management of root-knot on pepper.

REFERENCES

1. Payakhapaab, S., D. Boonyakiat and M. Nikornpun, 2012. Genetic evaluation and physico-chemical properties of chilies (*Capsicum annuum* L.). J. Agric. Sci., 4: 253-260.
2. Hershenhorn, J., Y. Goldwasser, D. Plakhine, G. Herzlinger, S. Golan, R. Russo and Y. Kleifeld, 1996. Role of pepper (*Capsicum annuum*) as a trap and catch crop for control of *Orobanche aegyptiaca* and *O. cernua*. Weed Sci., 44: 948-951.
3. Thiyagarajan, S.S. and H. Kuppasamy, 2014. Biological control of root knot nematodes in chillies through *Pseudomonas fluorescens*'s antagonistic mechanism. J. Plant Sci., 2: 152-158.
4. Jonathan, E.I., A. Sandeep, I. Cannayane and R. Umamaheswari, 2006. Bioefficacy of *Pseudomonas fluorescens* on *Meloidogyne incognita* in banana. Nematologia Mediterr., 34: 19-25.
5. Senthilkumar, T. and S. Ramakrishnan, 2004. Studies on compatibility of *Pseudomonas fluorescens*, *Trichoderma viride* and carbofuran 3G and their influences on *Meloidogyne incognita* in okra. Ann. Plant Prot. Sci., 12: 140-142.
6. Schneider, S.M., E.N. Roskopf, J.G. Leesch, D.O. Chellemi, C.T. Bull and M. Mazzola, 2003. United States Department of Agriculture-Agricultural Research Service research on alternatives to methyl bromide: Pre-plant and post-harvest. Pest Manage. Sci., 59: 814-826.
7. Crawford, J.M. and J. Clardy, 2011. Bacterial symbionts and natural products. Chem. Commun., 47: 7559-7566.
8. Norabadi, M.T., N. Sahebani and H.R. Etebarian, 2014. Biological control of root-knot nematode (*Meloidogyne javanica*) disease by *Pseudomonas fluorescens* (Chao). Arch. Phytopathol. Plant Prot., 47: 615-621.
9. Tian, B., J. Yang, L. Lian, C. Wang, N. Li and K.Q. Zhang, 2007. Role of an extracellular neutral protease in infection against nematodes by *Brevibacillus laterosporus* strain G4. Appl. Microbiol. Biotechnol., 74: 372-380.
10. Kerry, B.R., 2000. Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-parasitic nematodes. Ann. Rev. Phytopathol., 38: 423-441.
11. Black, C.A., ASA and ASTM, 1965. Methods of Soil Analysis: Part 1: Physical and Mineralogical Properties, Including Statistics of Measurement and Sampling. American Society Agronomy, Madison, Wisconsin, Pages: 770.
12. Mehlich, A., 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun. Soil Sci. Plant Anal., 15: 1409-1416.
13. Abdel-Baset, S., E. Abdelrazik and A. Shehata, 2020. Potentials of potassium humate, ammonium humate, and vermicompost tea in controlling root-knot nematode, *Meloidogyne arenaria* and improving biochemical components in eggplant. Afr. J. Biol. Sci., 16: 119-134.
14. Whitehead, A.G. and J.R. Hemming, 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann. Appl. Biol., 55: 25-38.
15. Sharma, P. and R. Pandey, 2009. Biological control of root-knot nematode; *Meloidogyne incognita* in the medicinal plant; *Withania somnifera* and effect of bio-control agents on plant growth. Afr. J. Agric. Res., 4: 564-567.
16. Adegbite, A.A. and G.O. Agbaje, 2007. Efficacy of Furan (Carbofuran) in control of root-knot nematode (*Meloidogyne incognita* race 2) in hybrid yam varieties in South-Western Nigeria. World J. Agric. Sci., 3: 256-262.

17. Li, X.Q., A. Tan, M. Voegtline, S. Bekele, C.S. Chen and R.V. Aroian, 2008. Expression of Cry5B protein from *Bacillus thuringiensis* in plant roots confers resistance to root-knot nematode. *Biol. Control*, 47: 97-102.
18. Oka, Y., 2010. Mechanisms of nematode suppression by organic soil amendments-A review. *Appl. Soil Ecol.*, 44: 101-115.
19. Riegel, C. and J.P. Noe, 2000. Chicken litter soil amendment effects on soilborne microbes and *Meloidogyne incognita* on cotton. *Plant Dis.*, 84: 1275-1281.