Plant Archives Vol. 25, Supplement 2, 2025 pp. 123-126 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-2.016

EFFECT OF NATIVE BACTERIAL ISOLATES AGAINST *MELOIDOGYNE INCOGNITA* IN PEA (*PISUM SATIVUM* L.)

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(Date of Receiving : 25-02-2025; Date of Acceptance : 30-04-2025)

ABSTRACT

Root-knot nematodes, *Meloidogyne incognita*, is one of the most frequently observed plant parasitic nematodes that causes severe yield losses. In this research, effect of different native bacterial isolates in pea against *M. incognita* was investigated during *rabi* seasons of 2023 –24. Native bacterial isolates namely *Bacillus megaterium* (MF36134), *B. amyloliquefacience* (OR304217), *B. subtilis* (OR298285), *Providencia rettgeri* (OR294194), *P. vermicola* (OR335551) and Carbofuran 3G as chemical check were used for these sick plot trials. The plant parameters such as shoot length, fresh and dry weight of shoot, root length, fresh and dry weight of root were found to be more in the plots treated with *B. subtilis* @ 0.8 l/ha followed by *B. amyloliquefacience* @ 0.8 l/ha as compared to untreated control. Carbofuran 3G @ 33 kg/ha was found to be very effective in reducing the egg masses per root system, eggs per egg mass, soil nematode population and root-knot index. Among the different bacterial isolates, *B. subtilis* (OR298285) exhibited the best result in reducing nematode population followed by *B. amyloliquefacience* (OR304217) as compared to untreated control. Highest yield was recorded in plants treated with *B. subtilis* (OR298285) followed by *B. amyloliquefacience* (OR304217). The above findings revealed that, *B. subtilis* (OR298285) showed antagonistic effect against *M. incognita* as compared to other native bacterial isolates.

Keywords **:** native bacterial isolates, *Meloidogyne incognita,* pea, *B. subtilis,* antagonistic.

Introduction

Pea (*Pisum sativum* L.) belonging to the legume family (Fabaceae) is one of the most cultivated vegetable crop grown in *rabi* season throughout the world. They are highly rich in starch, fiber, protein, vitamin A, vitamin B, vitamin C, vitamin K, phosphorus, magnesium, copper, iron, zinc and lutein (Pownall *et al*., 2010). Globally, it is cultivated over 5.9 million hectares with a production of about 11.7 million tons (Singh, 1983). In India, it is grown over 567 thousand hectares yielding about 5846 million tonnes (GoI, 2021). In Manipur, it is cultivated over an area of 7.51 thousand hectares with a production of 7643 tonnes in the year 2020-21 (GoI, 2021). Among various biotic stress attacking pea plants, the root-knot nematodes, *Meloidogyne incognita*, is one of the most important one that causes severe yield losses (Anwar and Mcknery, 2010) and has been reported to cause yield losses of up to 20 - 56 per cent in pea (De *et al*., 2000). Plant-parasitic nematodes (PPNs) are known to be the most destructive groups of crop pest (Trudgill and Blok, 2001), and causing an annual crop loss of approximately $125 billion globally (Chitwood, 2003). Chemical nematicides are also commonly used to manage root-knot nematodes, but these toxic chemicals pose a significant threat to the soil ecosystem and human health (Oka, 2010). So, the development of affordable and environmentally friendly bio-agents has become imperative due to restrictions on the use of

nematicides. *Bacillus* spp. such as *B. mycoides*, *B. subtilis*, *B. velezensis* and *B. pasteurii* were reported to reduce severity of root-knot nematode infestation and induce host resistance (Kloepper *et al*., 2004). *Bacillus* spp. is considered to be one of the important bacterial genera which plays crucial role in the management of nematodes (Kloepper and Ryu, 2006). This study aims to know the efficacy of native bacterial isolates against *M. incogita* in pea for sustainable agriculture.

Materials and Methods

Maintenance of pure culture and mass culturing of bacterial isolates: The five native bacterial isolates *viz*., *Bacillus megaterium* (MF36134), *B. amyloliquefacience* (OR304217), *B. subtilis* (OR298285), *Providencia rettgeri* (OR294194) and *P. vermicola* (OR335551) were maintained throughout the period of investigation. Pure cultures of these five bacterial isolates were also maintained and stored at 4°C in refrigerator. They were sub-cultured on fresh media (nutrient agar) and maintained in the BOD incubator. Mass culturing was done in nutrient broth and stored at BOD incubator for 3 days for multiplication. (colony forming unit of bacterial isolates = 1 x 10^8)

Field experiments were carried out in the research trial field, Department of Plant Pathology, College of Agriculture, CAU, Imphal having latitude of 24° 81$^{|}$N, longitude of 93°89$^{|}$E and an elevation of 790 metres above the mean sea level. The field experiment was carried out in Randomized Block Design (RBD) with 7 treatments and 4 replications. Pea seeds (var. Arkel) was sown in the already *M. incognita* infested field with a spacing of 30cm×15cm having plot size of 2m×2m.

The field experiment was conducted to study the antagonistic effect of *Bacillus megaterium, B. amyloliquefacience, B. subtilis*, *Providencia rettgeri*, *P. vermicola* and carbofuran 3G (chemical check) against *M. incognita* and the untreated plots served as control. Initial nematode population was recorded after land preparation. All the bio-agents and Carbofuran 3G (chemical check) were applied at the time of sowing as soil drenching. The bacterial isolates were applied @ 0.8 l/ha (concentration was diluted @10 ml/L of water). Ten plants from each plot were uprooted randomly. Plant parameters namely shoot length, root length, fresh and dry weight of shoots and roots were recorded. Number of galls per root system (Root-knot index, Taylor and Sasser, 1978), number of egg masses per root system and number of eggs per egg masses were also recorded. Nematode extraction from the soil for initial and final nematode population count was done using Cobb's Modified Sieving and Decanting Technique (Christie and Perry, 1951).

Root-knot index (Taylor and Sasser, 1978)

No. of galls per plant	Scale	Reaction
0	1	Highly resistant
1-10	2	Resistant
11-30	3	Moderately susceptible
31-100	4	Susceptible
> 100	5	Highly susceptible

Statistical Analysis

The data recorded for the different parameters were analyzed using the Analysis of Variance (ANOVA) method. The significance of the data collected was calculated by comparing the calculated "F" table value with the tabulated value of "F" at 5 per cent (5 %) level of probability. The field experiment was carried out in Randomized Block Design (RBD) with 4 replications and 7 treatments.

Results and Discussion

Present investigation for the year 2023-2024, the maximum shoot length (42.83 cm), root length (14.56 cm), fresh shoot weight (31.98 gm), dry shoot weight (4.38 gm), fresh root weight (6.20 gm) and dry root weight (1.68 gm) was recorded in the plants treated with *B. subtilis* (Table 1).

In terms of nematode parameters, minimum number of egg masses per root system (10.10), final nematode population (161.75), number of galls formed per root system (12.25) was observed Carbofuran 3G@ 33kg/ha treated plot. But among the native bacterial isolates, minimum number of egg masses per root system (12.55), final nematode population (194.50), number of galls formed per root system (15.75) was observed in T_3 which is treated with *B. subtilis* @ 0.8 l/ha (Table 2).

The results are in agreement with El-Nagdi *et al*. 2018, who showed that *B. subtilis*, *B. pumilus* (BP1), *B. pumilus* (BP2) when applied in soil, significantly increased the growth parameters of pea plants and reduced the *M. incognita* parameters. Vetrivelkalai (2019) also reported that, culture filtrates of two Bacillus spp. isolates *viz*., EB16, EB18, *Methylobacterium* spp., and one isolate of *Pseudomonas* sp. significantly reduced the number of adult females, egg masses, soil and root population of *M. incognita* in tomato on pot culture condition. Nyodu and Das (2020) reported that, the maximum plant growth parameters and minimum number of galls, egg mass per root system, final soil nematode population

were observed in tomato (var. Pusa Ruby) when treated with *P. fluorescens* @ 20g/kg seed followed by seed treatment with *B. subtilis* (vermi formulation) @20g/kg seed.

Table 1 : Effect of native bacterial isolates against *M. incognita* on different plant growth parameters of pea (2023-2024)
(Mean of 4 replications; Bacterial isolates were diluted @ 10 ml/l of water)

Treatments	Shoot Length (cm)	Shoot		Root Length (cm)	Root	
		Fresh Weight (gm)	Dry Weight (gm)		Fresh Weight (gm)	Dry Weight (gm)
T_1 = Soil application of *Bacillus megaterium* @ 0.8 l/ha	34.95	20.75	2.65	9.52	3.50 (0.65)	0.81 (0.26)
T_2 = Soil application of *B. amyloliquefacience* @ 0.8 l/ha	38.25	25.80	3.47	11.93	4.55 (0.74)	1.26 (0.35)
T_3 = Soil application of *B. subtilis* @ 0.8 l/ha	42.83	31.98	4.38	14.56	6.20 (0.86)	1.68 (0.43)
T_4 = Soil application of *Providencia rettgeri* @ 0.8 l/ha	28.75	15.75	1.98	8.85	2.60 (0.54)	0.60 (0.20)
T_5 = Soil application of *P. vermicola* @ 0.8 l/ha	32.23	16.25	2.10	9.15	2.75 (0.57)	0.67 (0.22)
T_6 = Carbofuran 3G @33 kg/ha (chemical check)	33.33	19.50	2.63	9.25	3.02 (0.60)	0.71 (0.23)
T_7 = Control	24.18	11.05	1.01	6.58	1.10 (0.32)	0.25 (0.09)
SE(d)±	1.53	1.91	0.35	0.93	0.04	0.03
CD (0.05)	3.21	4.02	0.73	1.96	0.09	0.07

*Figures in parenthesis are log transformed values

Table 2 : Effect of native bacterial isolates against *M. incognita* on nematode population in pea (2023-2024)
(Mean of 4 replications; Bacterial isolates were diluted @ 10 ml/l of water)

Treatments	No. of egg masses per root system	No of eggs per egg mass	Nematode population		Rf	No of galls/ root system	RKI	Yield		ICBR
			Initial	Final				(kg /plot)	(q/ha)	
T_1 = Soil application of *Bacillus megaterium* @ 0.8 l/ha	17.73	152.00	312.65	233.50	0.75	24.50	3.50	2.37	59.25	1: 2.55
T_2 = Soil application of *B. amyloliquefacience* @ 0.8 l/ha	15.83	143.50	336.72	225.25	0.67	21.25	3.25	2.43	60.75	1: 3.10
T_3 = Soil application of *B. subtilis* @ 0.8 l/ha	12.55	125.00	352.22	194.50	0.55	15.75	3.00	2.49	62.25	1: 3.65
T_4 = Soil application of *Providencia rettgeri* @ 0.8 l/ha	20.50	181.75	346.45	302.50	0.87	34.50	4.00	2.21	55.25	1: 1.10
T_5 = Soil application of *P. vermicola* @ 0.8 l/ha	19.25	166.75	325.38	284.50	0.87	31.50	3.75	2.26	56.50	1: 1.55
T_6 = Carbofuran 3G @33 kg/ha	10.10	106.75	379.15	161.75	0.43	12.25	2.75	2.35	58.75	1: 1.06
T_7 = Control	39.75	212.00	359.27	493.50	1.37	89.25	4.25	1.98	49.50	-
SE(d)±	1.02	6.06	-	11.76	-	-	-	0.07	0.53	-
CD (0.05)	2.13	12.73	-	24.72	-	-	-	0.14	1.12	-

Conclusion

The above findings resulted that the efficacy of bacterial isolates against *M. incognita* under field condition gave positive response. However, trials should be done under field conditions at different places to evaluate the accuracy or the effectiveness of the bio-control agents. Again, further evaluation of the bio-agents can be taken up at different level of doses and the mode of application under field condition.

Acknowledgement

The authors highly acknowledged the Department of Plant Pathology, College of Agriculture, CAU, Imphal and AICRP-Nematodes, Imphal Centre for

allowing us to perform the research work without any hindrance.

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