



RESEARCH ARTICLE

Biocontrol potential of *Bacillus megaterium* liquid bioformulation on *Meloidogyne incognita* infecting brinjal under field conditions

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Abstract

Brinjal (*Solanum melongena* L.) is one of the major vegetable crops grown in India. It is a low-calorie vegetable with high nutritional value due to the abundance of many components like fibers, proteins, phenolics, vitamins and minerals. However, its production is severely hampered by the incidence of major pests and diseases, including root-knot nematode. Root-knot nematode management is a challenge since nematicides have been mostly banned/restricted and resistant varieties or non-host crops are often not available. Therefore, plant growth-promoting rhizobacteria emerge safe and alternative option for nematode management. Thus, in the present study, *Bacillus megaterium* (1% A.S.) was evaluated through different application methods and its combination to decipher nematicidal efficacy against *Meloidogyne incognita* infecting brinjal under field conditions. Three years experimental results revealed that the treatment comprising of seed treatment with *Bacillus megaterium* (1% A.S.) at 10 mL/kg seed+ Nursery bed treatment with *Bacillus megaterium* (1% A.S.) 50 mL/m² + application of 5 tons of FYM enriched with 5.0 liter of *Bacillus megaterium* (1% A.S.) /ha + Soil drenching with *Bacillus megaterium* (1% A.S.) at 5 mL/liter at 30 days interval) significantly reduced *M. incognita* egg mass production per root system (81.7%), final nematode population in soil (60%), with least root gall index (1.0) and enhanced 20.7 of brinjal yield under field conditions. The present study elucidates that *B. megaterium* (1% A.S.) bioformulation and its combination of application methods could be one of the components in the integrated management of *M. incognita* infecting brinjal under field conditions.

Keywords: Brinjal, *Meloidogyne incognita*, *Bacillus megaterium*, Liquid bioformulation, Field evaluation.

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Introduction

Brinjal (*Solanum melongena* L.) is one of the major vegetable crops grown in India. It is presumed to be an Indigenous crop to India and China as a secondary center of origin (Chinthagunti et al., 2018). It is a low-calorie vegetable with high nutritional value due to the abundance of many components like fibers, proteins, phenolics, vitamins and essential minerals such as phosphorus, potassium, calcium as well as magnesium (Raigón et al., 2008; Akanksha et al., 2023). In India, brinjal is cultivated in an area of 0.675 million ha with a production of 12.76 million tonnes (FAOSTAT, 2022). However, brinjal production was severely hampered by the direct interference of plant parasitic nematodes (PPNs) with the plant root system. Among them, root-knot nematode (*Meloidogyne* spp.) is posing a serious threat to the cultivation of brinjal across the country. *Meloidogyne incognita* is the most frequently observed species among the *Meloidogyne* genus and causes about 21% annual yield loss with an estimated monetary loss of up to 3499.12 million rupees (Kumar et al., 2020).

The management of nematodes is largely dependent on chemical nematicides; however, due to their potential

negative impact on the environment, human health leads to phasing out many effective chemical nematicides. Thus search for alternative nematode management strategies became necessary. Biological control agents (BCA) have emerged as safe, cost-effective and environmentally friendly alternatives to chemical nematicides (Collange *et al.*, 2011; Thondihalu and Chawla, 2024).

In recent years, plant growth-promoting rhizobacteria (PGPR), i.e., *Bacillus* spp., has been recognized as one of the most promising candidates for the biocontrol of RKN (Engelbrecht *et al.*, 2018). They are ubiquitous within the rhizosphere, promote plant growth, and produce highly resistant endospores and produce a plethora of secondary metabolites, including lipopeptides, polyketides, lantibiotics, lytic enzymes and volatile compounds (Engelbrecht *et al.*, 2018; Caulier *et al.*, 2019). Among *Bacillus* spp., i.e., many native strains of *Bacillus subtilis*, *B. licheniformis*, *B. amyloliquefaciens*, *B. firmus*, *B. megaterium*, *B. pumilus*, *B. cereus* etc., are tested for their bio-efficacy against RKNs (Li *et al.*, 2007; Abdel-Salam *et al.*, 2018; Basyony and Abo-Zaid, 2018; Gowda *et al.*, 2018; Mhatre *et al.*, 2019; Gowda *et al.*, 2022; Gowda *et al.*, 2023). Thus, in the present investigation, the liquid bioformulation of *Bacillus megaterium* (1% A.S.) with different application methods was evaluated for the management of root-knot nematode *M. incognita* in brinjal under field conditions.

Materials and Methods

Experimental site

The field experiments were conducted in brinjal (cv. Kashi Taru) at a research farm (25°11'7.23"N, 82°E52'10.92"E), ICAR-Indian Institute of Vegetable Research, Varanasi, (Uttar Pradesh) for three consecutive years (2018, 2022 and 2023), during *Kharif* season. The study area is located in the alluvial zone of the Indo-Gangetic plain, which has a silt loam soil texture and pH of 7.3, which is neutral to slightly alkaline in reactivity. The soil also has an electrical conductivity of 0.31 dS/m. The root-knot nematode (*Meloidogyne incognita*) was prevalent in the nematology experimental site and was identified based on a perineal pattern of adult females (n=30).

Liquid bioformulation

Liquid bioformulation of *Bacillus megaterium* (1% A.S.) was procured from the Division of Entomology and Nematology, ICAR-Indian Institute of Horticultural Research, Bengaluru, for the present study.

Application methods

Seed treatment

Seed treatment for seed treatment, one kg of brinjal (cv. Kashi Taru) seeds was treated with 10 ml of *B. megaterium* (1% A.S.) bioformulation for 1 h. Then shade dried for 12 h before sowing in nursery beds.

Nursery drenching

The nursery bed was treated with *B. megaterium* (1% A.S.) at 50 ml/m²

Soil application

Liquid bioformulation of *B. megaterium* (1% A.S.) at 5 litre/ha was thoroughly mixed with farm yard manure (5 tonnes/ha) and then covered with polyethylene sheet by maintaining optimum moisture conditions under shade for 21 days. Further, enriched FYM was applied to respective treatments before transplanting.

Soil drenching

Soil drenching with *B. megaterium* (1% A.S.) 5 mL/litre at 30 days interval.

Field evaluation of bioformulation *Bacillus megaterium* (1% A.S.) against *Meloidogyne incognita* infecting brinjal

To evaluate the biocontrol potential of bioformulation *B. megaterium* (1% A.S.) against *M. incognita* a field experiment was laid out in randomized block design (RBD) with seven treatments, including different application methods. The treatments were as follows; T1-Seed treatment with *B. megaterium* (1% A.S.) at 10 mL/kg seed; T2- T1+ Nursery bed treatment with *B. megaterium* (1% A.S.) at 50ml/m²; T3- T2+ Soil drenching with *B. megaterium* (1% A.S.) at 5 mL/liter at 30 days interval; T4- T2+ application of 5 tonnes of FYM enriched with 5.0 liter of *B. megaterium* (1% A.S.) / ha; T5-T2+ application of 5 tonnes of FYM enriched with 5.0 liter of *B. megaterium* (1% A.S.) /ha + Soil drenching with *B. megaterium* (1% A.S.) at 5 mL/liter at 30 days interval; T6- Chemical treatment (Carbofuran at 1 kg a.i./ha); T7- Control. The crop was raised following standard agronomic practices. At the time of harvest, observations were recorded on nematode parameters such as gall on a 0 to 5 scale (Hussey and Janssen 2002). The final nematode population in soil was assessed by using Cobb's sieving and decanting method and modified Baermann technique (Cobb 1918; Walker and Wilson, 1960.). The number of egg masses per root system of each treatment was counted with the help of a magnifying glass and the marketable yield of brinjal recorded tonnes/ha.

Statistical Analysis

Analysis of variance (ANOVA) was performed for the numerical data on gall index, number of egg mass per root system and final nematode population in soil and brinjal yield using WASP-Web Agri Stat Package 2.0 (2004). The significant differences among the treatments ($p < 0.05$) were determined at the 5% significance level.

Results and Discussion

The present investigation found that *B. megaterium* (1% A.S.) liquid bio-bioformulation applied through different application methods exhibited nematicidal efficacy

against *M. incognita* and improved brinjal yield under field conditions. Among the treatments, the treatment (T5) comprising of seed treatment with *B. megaterium* (1% A.S.) at 10 mL/kg seed+ Nursery bed treatment with *B. megaterium* (1% A.S.) 50 ml/m² + application of 5 tonnes of FYM enriched with 5.0 litre of *B. megaterium* (1% A.S.) / ha + Soil drenching with *B. megaterium* (1% A.S.) at 5 mL/litre at 30 days interval) significantly suppressed the *M. incognita* incidence in brinjal by reducing the egg mass production per root system (78.0, 81.5, and 85.3%), final nematode population in soil (47.8, 64.7, and 67.7%), with least root gall index (2.2, 1.8, 1.7) during 2018, 2022, and 2023 in comparison to the control (Table 1). Furthermore, nematicide treatment (Carbofuran at 1 kg a.i./ha) reduced egg mass production per root system (67.2, 70.2, and 80.8%), final nematode population in soil (43.3, 54.1, and 56.5%) gall index (2.6, 2.2, 2.1) during 2018, 2022, and 2023 in comparison to the control (Table 1). The three years of pooled data revealed that, among the treatments, the treatment (T5) comprising of seed treatment with *B. megaterium* (1% A.S.) at 10 mL/kg seed+ Nursery bed treatment with *B. megaterium* (1% A.S.) 50 ml/m² + application of 5 tons of FYM enriched with 5.0 liter of *B. megaterium* (1% A.S.) /ha + Soil drenching with *B. megaterium* (1% A.S.) at 5 mL/liter at 30 days interval) found significantly effective in reducing *M. incognita* incidence in brinjal by reducing nematode reproduction (Table??).

Analysis of variance (ANOVA) showed that treatment T5 treatment was significantly ($p < 0.005$) differed from treatments T1, T2, T3, T4 and T7 in terms of all nematode reproduction parameters. However, the treatment T5 significantly ($p < 0.005$) differed with the reduction of egg mass production and gall index except for the final nematode population in soil with chemical treatment, i.e., Carbofuran at 1 kg a.i. /ha (Table 1). Furthermore, the brinjal yield (16.8, 23.4, and 23.0%) significantly enhanced in fields treated with bioformulation of *B. megaterium* (1% A.S.) with a combination of application methods during 2018, 2022, and 2023 (Fig. 1). analysis of variance showed that treatment T5 was found to be considerably superior to other treatments for the enhancement of brinjal yield (Fig. 1).

In the present investigation, *B. megaterium* (1% A.S.) bioformulation showed greater biocontrol potential against *M. incognita* infecting brinjal under field conditions. In a previous study, Padgham and Sikora (2007) revealed that the *B. megaterium*-treated rice roots showed more than 40% reduction in nematode penetration and gall formation as compared to non-treated rice roots. Similarly way, *B. megaterium* treatment reduced 50% of *Meloidogyne chitwoodi* and *Pratylenchus penetrans* invasion in potatoes (Al-Rehiyani *et al.*, 1999). Besides, in the present study, the combination of application methods, i.e., seed treatment, nursery drench, soil application and soil drenching at 30 days interval, protected brinjal crop from *M. incognita* infestation for the entire crop season by enhancing nematicidal efficacy and also consistency of *B. megaterium* under field conditions. In previous study demonstrated that, the incorporation of organic matter enhanced the efficacy of *B. megaterium* in soil and initiated antibiosis for nematode suppression (Mostafa *et al.* 2014).

In summary, the present study demonstrates that the application of *B. megaterium* bioformulation (1% A.S.) in combination of seed treatment, nursery drench, soil application of enriched FYM and soil drenching at 30-day intervals significantly protects brinjal crop from *M. incognita* incidence under field conditions. Thus, *B. megaterium* (1% A.S.) bioformulation and its combination of application methods could be one of the component in the integrated management of *M. incognita* in brinjal under field conditions.

Different letters on the top of error bars indicate statistically significant differences between treatments. A. 2018 B. 2022 C. 2023 field experiments Treatment details: T1-Seed treatment with *B. megaterium* (1% A.S.) at 10 mL/kg seed; T2- T1+ Nursery bed treatment with *B. megaterium* (1% A.S.) at 50 mL/m²; T3- T2+ Soil drenching with *B. megaterium* (1% A.S.) at 5 mL/litre at 30 days interval; T4- T2+ application of 5 tonnes of FYM enriched with 5.0 litre of *B. megaterium* (1% A.S.) /ha; T5-T2+ application of 5 tonnes of FYM enriched with 5.0 litre of *B. megaterium* (1% A.S.) /ha + Soil drenching with *B. megaterium* (1% A.S.) at 5 mL/litre at 30 days interval; T6- Chemical treatment (Carbofuran at 1 kg a.i./ha); T7- Control.

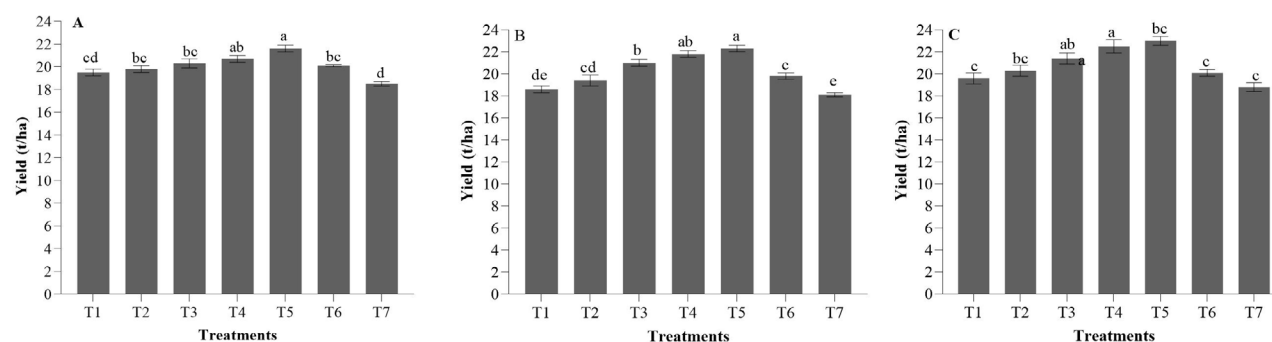


Fig. 1: Effect of *Bacillus megaterium* (1% A.S.) bioformulation on brinjal yield

Table 1: Nematicidal efficacy of *B. megaterium* (1% A.S.) bioformulation on *Meloidogyne incognita* infecting brinjal under field conditions

Treatments	Rki (0–5)				Egg mass per root system				Fnp (100cc)				Rf			
	2018		2022		2018		2022		2018		2022		2018		2022	
	Pooled Average		Pooled Average		Pooled Average		Pooled Average		Pooled Average		Pooled Average		Pooled Average		Pooled Average	
T1	3.9 ^a	3.8 ^a	4.0 ^a	3.9 ^a	167.7 ^b (-22.1)	152.7 ^b (-25.1)	156.2 ^b (-29.8)	158.8 ^b (-25.7)	413.3 ^a (-7.5)	380.0 ^a (-6.5)	399.0 ^b (-12.0)	397.4 ^b (-8.8)	2.1	2.5	2.3	2.3
T2	3.8 ^a	3.4 ^b	3.3 ^b	3.5 ^b	151.3 ^c (-29.7)	129.3 ^c (-36.5)	138.8 ^c (-37.6)	139.8 ^c (-34.6)	393.3 ^a (-11.9)	366.7 ^a (-9.8)	381.2 ^b (-15.9)	380.4 ^b (-12.7)	2.0	2.4	2.2	2.2
T3	3.1 ^b	2.6 ^c	2.6 ^c	2.8 ^c	104.3 ^d (-51.5)	97.7 ^d (-52.1)	113.7 ^d (-48.9)	105.2 ^d (-50.8)	320.0 ^b (-28.4)	283.3 ^b (-30.3)	272.1 ^c (-40.0)	291.8 ^c (-33.0)	1.7	1.8	1.6	1.7
T4	2.8 ^c	2.8 ^c	2.7 ^c	2.7 ^c	95.3 ^d (-55.7)	90.3 ^d (-55.7)	89.0 ^e (-60.0)	91.6 ^d (-57.2)	293.3 ^b (-34.3)	270.0 ^b (-33.6)	252.1 ^c (-44.4)	271.8 ^c (-37.6)	1.5	1.8	1.5	1.6
T5	2.2 ^d	1.8 ^e	1.7 ^e	1.9 ^e	47.3 ^f (-78.0)	37.7 ^f (-81.5)	32.6 ^g (-85.3)	39.2 ^f (-81.7)	233.3 ^c (-47.8)	143.3 ^c (-64.7)	146.5 ^e (-67.7)	174.4 ^d (-60.0)	1.2	0.9	0.9	1.0
T6	2.6 ^c	2.2 ^d	2.1 ^d	2.3 ^d	70.7 ^e (-67.2)	60.7 ^e (-70.2)	42.7 ^f (-80.8)	58.0 ^e (-72.9)	253.3 ^c (-43.3)	186.7 ^c (-54.1)	197.0 ^d (-56.5)	212.3 ^d (-51.3)	1.3	1.2	1.2	1.2
T7	4.0 ^a	4.0 ^a	4.1 ^a	4.0 ^a	215.3 ^a (0.0)	203.7 ^a (0.0)	222.4 ^a (0.0)	213.8 ^a (0.0)	446.7 ^a (0.0)	406.7 ^a (0.0)	453.3 ^a (0.0)	435.6 ^a (0.0)	2.3	2.7	2.7	2.5
Cd (0.05)	0.27	0.34	0.25	0.36	12.66	12.04	8.15	13.87	63.49	46.34	29.07	51.44	--	-	-	-

Different letters on each column indicate statistically significant difference between treatments. (-) = Percent decrease over control, Gi: Gall index, FNP: Final nematode population in soil, RF: Reproduction factor.

Treatment details: T1-Seed treatment with *B. megaterium* (1% A.S.) at 10 ml/kg seed; T2- T1+Nursery bed treatment with *B. megaterium* (1% A.S.) at 50ml/m²; T3- T2+ Soil drenching with *B. megaterium* (1% A.S.) at 5 ml/litre at 30 days interval; T4- T2+ application of 5 tonnes of FYM enriched with 5.0 litre of *B. megaterium* (1% A.S.) /ha; T5-T2+ application of 5 tonnes of FYM enriched with 5.0 litre of *B. megaterium* (1% A.S.) /ha + Soil drenching with *B. megaterium* (1% A.S.) at 5 ml/ litre at 30 days interval; T6- Chemical treatment (Carbofuran at 1 kg a.i./ha); T7- Control.

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सारांश

बैंगन की खेती में जड़- गांठ सूत्रकृमी सहित प्रमुख कीटों और बिमारियों के प्रकोप इसका उत्पादन गंभीर रूप से बाधित होता है। वर्तमान अध्ययन में, बैसिलस मेगाटेरियम (1% ए.एस.) का मूल्यांकन विभिन्न अनुप्रयोग विधियों और इसके संयोजन के माध्यम से किया गया ताकि क्षेत्रीय परिस्थितियों में बैंगन को संक्रमित करने वाले मेलोइडोगाइन इन्कोग्निटा के विरुद्ध की सूत्रकृमिनाशकों प्रभावकारिता का पता लगाया जा सके। तीन वर्षों के प्रायोगिक परिणामों से पता चला है कि 10 मिली/किलोग्राम बीज की दर से बैसिलस मेगाटेरियम (1% ए.एस.) से बीज उपचार + 50 पौधशाला मिली/वर्ग मीटर बैसिलस मेगाटेरियम (1% ए.एस.) से पौधशाला उपचार 5.0 लीटर बैसिलस मेगाटेरियम (1% ए.एस.) से समृद्ध 5 टन गोबर की खाद/हेक्टेयर का प्रयोग 30 दिनों के अंतराल पर 5 मिली/लीटर बैसिलस मेगाटेरियम (1% ए.एस.) से मिट्टी को गीला करने से प्रति जड़ प्रणाली में मेलोइडोगाइन इन्कोग्निटा अंडे का उत्पादन (81.7%), मिट्टी में अंतिम सूत्रकृमि की संख्या (60%), न्यूनतम रूट गॉल इंडेक्स (1.0) में उल्लेखनीय कमी आई और क्षेत्रीय स्थितियों में बैंगन की उपज में 20.7% की वृद्धि हुई। वर्तमान अध्ययन स्पष्ट करता है कि बैसिलस मेगाटेरियम (1% ए.एस.) जैवसूत्रीकरण और इसके अनुप्रयोग विधियों का संयोजन क्षेत्रीय परिस्थितियों में बैंगन को संक्रमित करने वाले मेलोइडोगाइन इन्कोग्निटा के एकीकृत प्रबंध में एक घटक हो सकता है।