

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.903.021>

Effect of bacterial antagonists on Root knot nematode, *Meloidogyne incognita* infecting brinjal

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ABSTRACT

Keywords

Pseudomonas fluorescens,
Bacillus subtilis,
Meloidogyne incognita, *Solanum melongena*,
Treatments

Article Info

Accepted:
05 February 2020
Available Online:
10 March 2020

A pot culture experiment was carried out in a net house condition of Department of Nematology, College of Agriculture, O.U.A.T, Bhubaneswar during 2017-2018 to assess the nematode control potential of *Pseudomonas fluorescens* and *Bacillus subtilis* on the growth of the brinjal plant var. Mukta Began and reduction of root-knot nematode, *Meloidogyne incognita*. The experiment was designed in complete randomised block design comprising of eight treatments with three replications of each treatment. Among all the treatments, it was revealed that T₆ (Soil application of *Bacillus subtilis* @3mg/kg) observed maximum increase in growth parameters like increase in plant height by (40.56%), fresh shoot weight (80.25%), fresh root weight (97.22%), dry shoot weight (97.11%), dry root weight (94.44%), root length (98.17%), root volume (65.23%) with decrease of number of nematode population in soil and root respectively, (70.64%) and (44.73%) over untreated check T₈.

Introduction

Brinjal (*Solanum melongena*) is popularly known as baigan which belongs to Solanaceae family. It is a delicate, tropical perennial vegetable often cultivated as a tender or half-hardy annual in temperate climates. It is one of the largest grown vegetable crop in the world. It is one of the most common tropical vegetables grown in India. India ranks second (12.6 million tonnes) in the production of brinjal. It is the

moderate source of vitamins and minerals like phosphorous, calcium, iron and nutritive value varies from variety to variety. In India Cultivation of brinjal is maximum in Orissa, West Bengal, Bihar and is also distributed in almost all states.

Meloidogyne incognita is a difficult crop pests to control (Chitwood *et al.*, 2002) as they have high reproduction rate. Root-knot nematodes are sedentary obligate endoparasitic nematodes, which common in

Egypt and worldwide and cause severe crop damage especially in light soils that cause major economic damage to crops. Root-knot nematodes (*Meloidogyne* spp.) cause serious damage on a wide range of crops, especially on vegetables such as tomato, potato, eggplants, okra etc. in tropical and subtropical agriculture (Anamika *et al.*, 2011). The young tender seedlings of various crops are very much vulnerable to attack by nematode while the older plants achieve some degree of tolerance. The easy, traditional and quick down effects of nematode control is mainly based on the use of chemical nematicides, yet the harmful effects on environment due to residual effects of toxic compounds, create health hazard in human, increase risk of pollution, imbalance between various eco-systems and pest resurgence after prolonged use.

So a classical biological approach, which may emphasize on the non-conventional method of nematode management by using cost effective sources like bio-control agents and organic amendments as eco- friendly alternatives to stabilize vegetable production. The notable biocontrol agents like *Pseudomonas fluorescens* and *Bacillus subtilis* are known to have a significant role in suppression of root-knot nematode diseases (Siddiqui *et al.*, 2002) and their effectiveness was reported by Dhawan and Singh, 2010. Therefore, the present experiment research work “Effect of bacterial antagonists on *Meloidogyne incognita* infecting brinjal” was concentrated to exploit effectiveness of bio management in suppression of *Meloidogyne incognita* infecting brinjal variety Mukta Began by using two BCAs i.e. *Pseudomonas fluorescens* and *Bacillus subtilis*.

Materials and Methods

The experiment was entirely conducted under pot culture condition in the net house of Dept

of Nematology during Dec 2017- May 2018 and following Complete Randomised Block Design (CRD).

Preparation of soil and pot

For experimental and culturing of nematode purpose, well pulverized sandy loam soil free from plant debris and gravels. The soil was mixed thoroughly with sand and FYM in the ratio of 2:1:1, which was filled in gunny bags and autoclaved at 1.1kg/cm² pressure for one hour daily for two consecutive days. The sterilized soil was spread on a clean polythene sheet for 24 hours for renaturation of soil. In the meantime the earthen pots of 15cm diameter were cleaned and surface sterilized in 1% formaldehyde solution and made air dry.

Sowing of seeds

Brinjal variety- Mukta began seeds were surface sterilized in 2.5% Sodium hypochlorite solution for 2 min. Three to four seeds were sown in each pot and lightly covered with sterilized soil. Seeds when germinated and attained 3-4 leaves stage, thinning was done keeping one plant per pot.

Nematode culture

Egg masses of *Meloidogyne incognita* were collected from brinjal roots, and the population was multiplied inoculating single egg mass culture on a susceptible brinjal variety grown in pots containing sterilized soil. This was done two months prior to the start of experiment.

Standardizing nematode number in stock solution

Freshly hatched J₂ of *Meloidogyne incognita* were isolated and surface sterilized by treating with aqueous solution of

Mercurochrome (0.1%) for 30 mins, in order to get rid of any bacterial and fungal contaminants. Then the juveniles were washed 4-5 times in sterilized distilled water. The nematode suspension containing J₂ was taken in a beaker.

Inoculation of nematodes

20 days after sowing of seeds in pots, infective J₂ of root knot nematode were inoculated @1000 J₂/kg soil. Two small holes of 2cm depth were made in the soil close to base of the plant, into which a measured volume of nematode suspension (20ml) was slowly poured to release 1000 J₂ per pot in all the treatments and close the holes immediately.

Treatments and recording of Observation

T₁. Soil application of *Pseudomonas fluorescens* @2.0 mg/kg soil, T₂. Soil application of *Pseudomonas fluorescens*@2.5 mg/kg soil, T₃. Soil application of *Pseudomonas fluorescens*@3 mg/kg soil, T₄. Soil application of *Bacillus subtilis* @ 2.0 mg/kg soil, T₅. Soil application of *Bacillus subtilis* @ 2.5 mg/kg soil, T₆. Soil application of *Bacillus subtilis* @ 3.0 mg/kg soil, T₇. Soil application of Carbofuran@ 0.5 mg a.i/kg soil, T₈. Uninoculated control.

Sixty days after sowing, each plant was removed from pot soil carefully. Roots are washed free from soil and other adhering particles under slow stream of water and observation was recorded on different plant growth parameters, nematode population in soil as well as root.

Results and Discussion

From the experiment data cited in Table-1, it was observed that there was significant increase in plant shoot length in all the treatments over the untreated check.

Maximum plant height 26.40 cm was recorded in the treatment T₆ with an increase of 40.56% over. The percentage of increase in plant height was followed in descending order as T₆(40.56%), T₅(34.95%), T₄(31.78%), T₇(26.36%), T₃(25.98%), T₂(15.14%) and T₁(10.47%). The significant increase in root length were observed as follows: T₁(11.98%), T₂(57.97%), T₃(66.39%), T₄(82.46%), T₅(89.01%), T₆(98.17%), T₇(69.56%) over untreated check T₈. All treatments were observed significantly superior over the check; but among all T₆ showed maximum root length of 35.33 cm.

In treatment T₆ recorded the maximum significant increase of fresh shoot weight(25.00g) than other treatments. Highest fresh shoot weight was recorded in T₆(80.25%) followed by T₅(72.31%), T₄(66.55%), T₇(64.86%), T₃(57.89%), T₂(11.75%) and T₁(10.31%) over check T₈. Fresh root weight in T₁ was 5.20g, T₂: 6.20g, T₃: 6.57g, T₄: 6.70g, T₅: 6.77g, T₆: 7.10g, T₇: 6.67g over T₈: 3.60g. Highest significant increase of fresh root weight was observed in T₆(97.22%).

The significant increase in dry shoot weight were calculated: T₁(30.03%), T₂(39.32%), T₃(42.41%), T₄(71.31%), T₅(94.01%), T₆(97.11%) and T₇(51.70%) over T₈. The mean dry shoot weight in T₆(6.37g) was found non-significantly at par with T₅(6.27g) but significantly higher than other treatments. The maximum dry root was recorded in T₆94.44% followed by T₅: 91.67%, T₄:77.78%, T₇:75%:, T₃:61.11%, T₂:38.89% and T₁:8.33% over T₈.

The percentage of significant increase in root volume of brinjal plant due to the various treatments were recorded in an ascending order T₁(26.16%), T₂(26.88%), T₃(30.11%), T₇(32.26%), T₄(36.56%), T₅(43.01%), T₆(65.23%) over T₈.

Table.1 Effects of bacterial antagonists as soil application against *Meloidogyne incognita* on crop growth parameters of brinjal cv. Mukta Began

Treatments	Plant height (cm)	% of increase or decrease over control	Root length (cm)	% of increase or decrease over control	Fresh shoot weight(g)	% of increase or decrease over control	Fresh root weight (g)	% of increase or decrease over control	Dry shoot weight (g)	% of increase or decrease over control	Dry root weight(g)	% of increase or decrease over control
T ₁ - Soil application of <i>Pseudomonas fluorescens</i> @ 2.0mg/kg	4.20	+30.03	1.30	+8.33	15.30	+10.31	5.20	+44.44	4.20	+30.03	1.30	+8.33
T ₂ - Soil application of <i>Pseudomonas fluorescens</i> @2.5mg/kg	4.50	+39.32	1.67	+38.89	15.50	+11.75	6.20	+72.22	4.50	+39.32	1.67	+38.89
T ₃ - Soil application of <i>Pseudomonas fluorescens</i> @3mg/kg	4.60	+42.41	1.93	+61.11	21.90	+57.89	6.57	+82.41	4.60	+42.41	1.93	+61.11
T ₄ - Soil application of <i>Bacillus subtilis</i> @2.0mg/kg	5.53	+71.31	2.13	+77.78	23.10	+66.55	6.70	+86.11	5.53	+71.31	2.13	+77.78
T ₅ - Soil application of <i>Bacillus subtilis</i> @2.5mg/kg	6.27	+94.01	2.30	+91.67	23.90	+72.31	6.77	+87.96	6.27	+94.01	2.30	+91.67
T ₆ - Soil application of <i>Bacillus subtilis</i> @3mg/kg	6.37	+97.11	2.33	+94.44	25.00	+80.25	7.10	+97.22	6.37	+97.11	2.33	+94.44
T ₇ - Soil application of carbofuran @0.5mg ai/kg	4.90	+51.70	2.10	+75.00	22.87	+64.86	6.67	+85.19	4.90	+51.70	2.10	+75.00
T ₈ - Untreated check	3.23		1.2		13.87		3.60		3.23		1.2	
SEm(±)	0.40		0.25		0.73		0.25		0.40		0.25	
CD _(0.05)	0.86		0.53		1.56		0.53		0.86		0.53	

T₁. Soil application of *Pseudomonas fluorescens* @2.0 mg/kg soil, T₂. Soil application of *Pseudomonas fluorescens*@2.5 mg/kg soil, T₃. Soil application of *Pseudomonas fluorescens*@3 mg/kg soil, T₄. Soil application of *Bacillus subtilis* @ 2.0 mg/kg soil, T₅. Soil application of *Bacillus subtilis* @ 2.5 mg/kg soil, T₆. Soil application of *Bacillus subtilis* @ 3.0 mg/kg soil, T₇. Soil application of Carbofuran 0.5 mg a.i/kg soil, T₈. Uninoculated control

Table.2 Effects of bacterial antagonists as soil application against *Meloidogyne incognita* on the infection parameters and its population growth in brinjal cv. Mukta Began

Treatments	Root volume(cc)	% of increase or decrease over control	Final nematode population in soil	% of increase or decrease over control	Final nematode population in root	% of increase or decrease over control
T ₁ - Soil application of <i>Pseudomonas fluorescens</i> @ 2.0mg/kg	11.73	+26.16	957.33 (2.98)	-50.22	965 (2.98)	-1.60
T ₂ - Soil application of <i>Pseudomonas fluorescens</i> @2.5mg/kg	11.8	+26.88	851.33 (2.93)	-55.73	853.67 (2.93)	-12.95
T ₃ - Soil application of <i>Pseudomonas fluorescens</i> @3mg/kg	12.1	+30.11	829.33 (2.92)	-56.87	837.00 (2.92)	-14.65
T ₄ - Soil application of <i>Bacillus subtilis</i> @2.0mg/kg	12.7	+36.56	729.00 (2.86)	-62.09	663.67 (2.82)	-32.33
T ₅ - Soil application of <i>Bacillus subtilis</i> @2.5mg/kg	13.3	+43.01	643.33 (2.80)	-66.55	618.67 (2.79)	-36.91
T ₆ - Soil application of <i>Bacillus subtilis</i> @3mg/kg	15.36	+ 65.23	564.67 (2.88)	-70.64	542.00 (2.73)	-44.73
T ₇ - Soil application of carbofuran @0.5mg ai/kg	12.3	+32.26	774.33 (2.89)	-59.73	746.00 (2.87)	-23.93
T ₈ - Untreated check	9.20		1933.00 (3.28)		980.67 (2.99)	
SEm(±)	0.71		0.03		0.01	
CD _(0.05)	1.50		0.07		0.30	

T₁. Soil application of *Pseudomonas fluorescens* @2.0 mg/kg soil, T₂. Soil application of *Pseudomonas fluorescens*@2.5 mg/kg soil, T₃. Soil application of *Pseudomonas fluorescens*@3 mg/kg soil, T₄. Soil application of *Bacillus subtilis* @ 2.0 mg/kg soil, T₅. Soil application of *Bacillus subtilis* @ 2.5 mg/kg soil, T₆. Soil application of *Bacillus subtilis* @ 3.0 mg/kg soil, T₇. Soil application of Carbofuran 0.5 mg a.i/kg soil, T₈. Uninoculated control

T₆ was non-significantly at par with the treatments T₅, T₄ & T₇, but greater significant differences with the treatments T₃, T₂ & T₁ as compared to the inoculated check T₈. T₆ was significantly superior than all other treatments over untreated control T₈.

Hence, the egg parasitic bacteria *Bacillus subtilis* caused maximum reduction of egg masses as well as juvenile & adult root knot nematode population in roots and soil, when applied.

The present study i.e. effect of bacterial antagonists on root knot nematode, *Meloidogyne incognita* infecting brinjal (var. Mukta began), use of bio-control agents with different measure along with a chemical nematicide carbofuran 3G exhibited significant increase in plant growth parameters of brinjal var. Mukta began by reducing *Meloidogyne incognita* population and other infection parameters in each treatments over inoculated check.

Highest percentage of increase in plant height, root length, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight, root volume and maximum reduction in nematode population in soil and in root.

Plant growth varied with the bacterial strains; *Bacillus coagulans* showed stimulatory effect on plant growth while the response to *Bacillus subtilis* was somewhat inhibitory to neutral. Surette *et al.*, (2003) tested a number of bacterial strains on a variety of crops and demonstrated that 10–38 % of strains were plant growth neutral while 7–29 % were plant growth inhibitory.

Won-Chan and Rhee (2012) showed that a strain of *B. subtilis* produces some allelochemicals that suppress growth hormones like gibberellins and thereby inhibit plant growth. *Bacillus* inoculated plants showed reduced penetration of root-knot nematode compared to infected control plants at both the time periods. Some saprotrophic species are known to penetrate the cuticle of nematodes, propagate rapidly, and cause degradation and digestion of nematode tissues by the involvement of hydrolytic enzymes (Huang *et al.*, 2005; Niu *et al.*, 2006). Down regulation of photosynthesis-related genes was found to be part of a defense or adaptive response to stress (Bilgin *et al.*, 2010).

Bacterial application in the rhizosphere may

promote superoxide dismutase activity. Plant growth promoting strain of *Pseudomonas* after inoculation increased the activity of superoxide dismutase in leaves of eggplant (Fu *et al.*, 2010). In recent study, *Bacillus subtilis* showed maximum inhibition of root-knot nematode parasitism. However, plant growth in term of height and fresh weight were found insignificant compared to root-knot nematode treated controls.

It seems that *Bacillus subtilis* produced some kind of metabolites which were toxic to root-knot nematode as well as for the brinjal. But among all the treatments, it was revealed that T₆(Soil application of *Bacillus subtilis* @3mg/kg) observed maximum increase in growth parameters like plant height, root length, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight, root volume with least nematode infection parameters.

After all the experimental work it was seen that all the treatments were significantly superior to untreated check T₈ with increase in growth parameters and reduction in population. However among all the treatments, it was revealed that T₆(Soil application of *Bacillus subtilis* @3mg/kg) observed maximum increase in growth parameters and reducing nematode growth in root and soil. Therefore application of *Bacillus subtilis* @3mg/kg of soil may be recommended for management of root knot nematode *M.incognita* in brinjal.

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How to cite this article:

Anwasha Acharya, Anukiran Sahu and Das. B. K. 2020 Effect of bacterial antagonists on Root knot nematode, *Meloidogyne incognita* infecting brinjal. *Int.J.Curr.Microbiol.App.Sci.* 9(03): 171-177. doi: <https://doi.org/10.20546/ijcmas.2020.903.021>