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Effect of native actinobacteria on root knot nematode, *Meloidogyne incognita* in brinjal

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Abstract
Root knot nematode, *Meloidogyne incognita* is one of the major nematodes infesting brinjal. It is wide spread in India. An attempt was made to exploit rhizosphere bioagents for the management of root knot nematode. Rhizosphere soil samples were collected from healthy brinjal plants of nematode infested sick field at Virinchipuram village, Tamil Nadu. Around six *Bacillus* sp. isolates and one actinobacteria isolate were obtained from soil. Preliminary screening was done with culture filtrates of these isolates on egg hatching and nematode penetration. The observations showed that actinobacteria (V5) cultural filtrate @ 75% concentration showed 51.7 % inhibition in egg hatching compared to control. A significant reduction in juvenile penetration ($t_c > t_t$) and number of galls per plant as well as number of egg masses were observed in actinobacteria treated brinjal (variety VRM1) plants. A pot culture experiment was carried out to test the effect actinobacteria (V5) in brinjal against *M. incognita*. Results revealed that the egg mass production was reduced to an extent of 55 % compared to untreated control by the application of actinobacteria culture filtrate.

Keywords: Brinjal, *Meloidogyne incognita*, native isolates, actinobacteria, hatching inhibition, penetration test and pot culture experiment

Introduction
Spiny brinjal is an important vegetable crop grown in Vellore district of Tamil Nadu. It has good market demand in Tamil Nadu, Andra Pradesh and Karnataka states. Incidence of root knot nematode was recorded from several parts of Tamil Nadu. Nematicides available in India are likely to be withdrawn in near future due to their hazardous effect hence, new alternates needs to be formulated to contain nematode damage. Biological control of root knot nematode was carried out several workers [1]. Incorporation of neem leaves along with biocontrol agents *Pochonia chlamydosporia*, *Purpureocillium lilacinum* and *Trichoderma asperellum* resulted in 17, 21 and 14 per cent increase in yield [2]. They also reported that the percentage of reduction in nematode infestation was lower when neem leaves and *P. chlamydosporia* applied together. The effect of different biocontrol agents against root knot nematode, *M. incognita* in brinjal was studied by [3] and they have concluded that the combination of *Paecilomyces lilacinus* @ 25 kg spore dust and carbofuran 3G @ 2 kg / ha in two equal splits improved plant growth and considerably reduced gall index. The combined application of chicken manure along with *T. harzianum* showed highest reduction of root knot nematode in brinjal [4]. Few pot culture studies were undertaken using avermectin which is a product of actinomycetes. Results obtained by [5] showed that combined effect avermectins along with *Syncephalastrum racemosum* reduced root knot nematode population and gall development which was 76.5% reduction over control. Based on these findings, an attempt was made to test the native isolates of *Bacillus* and actinobacteria against root knot nematode. The methods adopted in study and results obtained are presented in this paper.

Materials and Methods
Isolation of bacterial and actinomycetes from brinjal rhizosphere soil
Native isolates of bacteria were obtained from brinjal rhizosphere soil using serial dilution technique. Soil samples were collected from rhizosphere region of brinjal plants. A healthy brinjal plant was selected from nematode infested field for collection of rhizosphere soil. Samples were weighed and mixed with sterile water blanks to isolate bioagents from soil. 1g of wet soil sample was mixed with 99ml sterile water blank to get 10^{-2} dilution and mixed thoroughly. 1 ml of this suspension was transferred to 9ml of sterile water to get 10^{-3}.

The same procedure was repeated till 10^{-4} dilution. 1ml of suspension from 10^{-3} was transferred to Petri plate and molten nutrient agar (NA) was poured. The plates were incubated under room temperature (29±2 ºC) for three days. Single colonies were subcultured in NA. Colonies which showed morphologically different were sub cultured for further studies.

Hatching study

Hatching and juvenile mortality tests were conducted with culture filtrates. Single egg mass was incubated in culture filtrate. Hatching and egg development were recorded. Number of juveniles hatched out was recorded at 24, 48 and 72h intervals. The activity of second stage juvenile in different culture filtrates were observed at 24 and 48h interval.

Effect of actinobacteria (V5) on *M. incognita* development

i) Hatching experiment

Based on the previous experiment one isolate was selected for hatching study. Various concentrations of culture filtrate *viz*., 25, 50 and 75% were prepared using sterile water. Single egg mass was incubated in 3ml of culture filtrate. Observation on number of juveniles hatched out was recorded at 24, 48, 72 and 120h.

ii) *In vitro* study on nematode penetration

An *in vitro* study was carried out to test the effect of actinobacteria (V5) on penetration of juveniles into brinjal roots. Sterile soil (red loamy) was filled in plastic cups (100g). Culture filtrate was mixed in soil and then brinjal seedlings (VRM1) were transplanted. Freshly hatched juveniles (1J2 / g soil) were inoculated into the cups for developmental study. Plants were uprooted carefully 7 days after inoculation and stained in acid fushcin (5g acid fushcin + 100ml lactophenol) lactophenol solution. Number juveniles penetrated were observed under research microscope (Leica model) after destaining with plain lactophenol.

Treatment details:

T1 - Control (sterile water)

T2 - Culture filtrate 50% (10ml / cup)

Treatments : 2 Replications : 13 Design : CRD - Paired t test

iii) Pot culture experiment

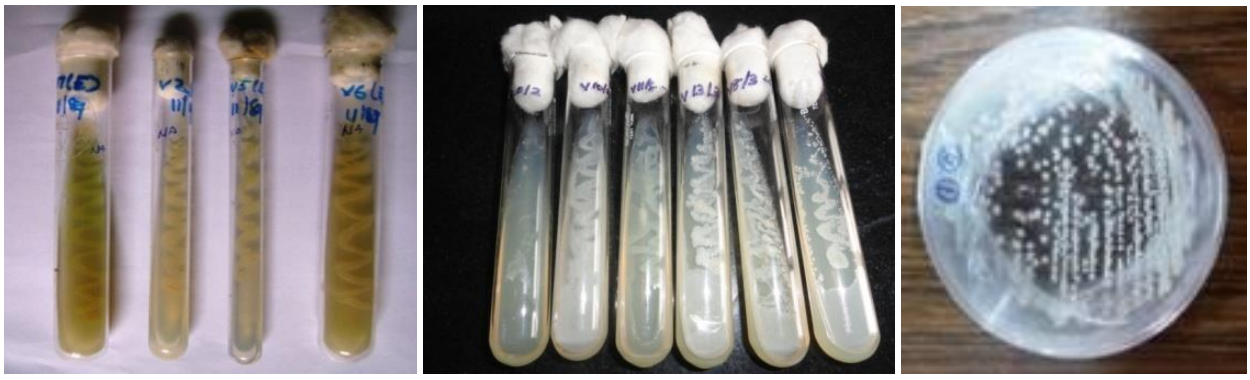
A pot culture experiment was undertaken at Agricultural Research Station, Virinjipuram, Tamil Nadu with spiny brinjal (VRM1). Pots were filled with sterilized pot mixture and seedlings of brinjal were planted. Different bioagents *viz*., actinobacteria (50% culture filtrate isolate V5), *P. lilacinum* @ 10g / pot and *T. asperallum* @ 10g / pot along with Carbofuran@ 3g / pot as positive check and untreated control. Bioagents were mixed with soil before transplanting. The second stage juveniles (1 J2/ g soil) were inoculated into the pots ten days after transplanting. Observation on nematode development was recorded 60 days after planting.

Treatments : 5 Replications : 4 Design : CRD

Results

Identification of native isolates

Soil samples were collected from brinjal rhizosphere to isolate native microbial antagonists. Seven different isolates were obtained from soil. Six of them resembled bacteria only one resembles actinobacteria. These isolates were subcultured and maintained for further research. The isolates were designated as V1, V2, V3, V4, V5, V6 and V7. Among them V1, V2, V3, V4, V6 and V7 were confirmed as *Bacillus* sp. based on morphological features. The isolate V5was identified as actinobacteria (Fig. 1).



Bacterial isolates Actinobacteria V5 isolate of actinobacteria

Fig 1: Native isolates of bacteria and actinobacteria

Hatching study

Root knot nematode egg masses were incubated in culture filtrates of native bacterial and actinobacteria (V5) for 48h. Egg development and hatching were recorded. Based on the observations, isolate V5 was selected as potential one (Table 1). All the isolates inhibited hatching but the isolate V5, completely inhibited the egg development. In contrast, egg development and hatching were normal under control (tap water).

Table 1: Effect of different isolates on *M. incognita* egg development

Isolates	Activity
V1 - *Bacillus* sp.	Cell division and egg development did not occur.
V2 - *Bacillus* sp.	Cell division observed, J1 was developed inside the egg but hatching was inhibited.
V3 - *Bacillus* sp.	J2 were hatched out but not active.
V5 - *Actinobacteria*	Egg development was completely stopped and no cell division occur within the egg but eggs disintegrated

	from gelatinous matrix.
V6 - *Bacillus* sp.	No cell division in eggs but eggs disintegrated from gelatinous matrix
V7 - *Bacillus* sp.	No cell division occur in eggs
Control (tap water)	75 percent eggs hatched out. J2 were live and active

Similarly highest inhibition of juvenile activity was recorded in culture filtrate of the actinobacteria isolate V5 (Table 2). Juvenile activity was observed at 24 and 48h intervals. All the isolates had influenced juvenile activity. More number of inactive juveniles were recorded in actinobacteria which was followed by bacterium, *Bacillus* sp. (V1). At 48h interval also, actinobacteria (V5) recorded with highest number of inactive juveniles.

Table 2: Effect of different isolates on *M. incognita* juvenile activity

Isolate	Second stage juvenile activity			
	24h		48h	
	Active	Inactive	Active	Inactive
V1	12.25	4.75	7.75	7.0
V2	11.75	3.0	8.25	7.0
V3	12.25	3.0	8.0	7.25
V4	13.15	1.75	10.5	5.75
V5	9.75	6.0	7.25	8.75
V6	13.0	2.75	10.25	5.75
V7	13.75	2.75	12.25	6.5
Control	14.25	1.25	12.5	3.5
CD (p=0.05)	4.83 (NS)	1.13	1.1	0.86

Effect of actinobacteria (V5) on *M. incognita* development

i) Hatching experiment

Effect actinobacteria culture filtrate was tested under laboratory condition. The observation showed that the highest inhibition of hatching was recorded under actinobacteria culture filtrate @ 75% concentration which was 51.7 per cent lesser compared to control at 120h of incubation (Table 3). Hatching was normal in tap water and little inhibition was recorded in positive check with plain Knight's broth.

Table 3: Effect of V5 (actinobacteria) on egg hatching

Concentration	24h	48h	72h	120h
	Number of juveniles hatched out			
T1 - 25 % culture filtrate	7.0	14.3	43.0	91.7
T2 -50 % culture filtrate	2.5	8.3	27.5	76.0
T3 -75 %culture filtrate	0.8	5.0	17.5	58.8
Control (Knight's broth)	4.7	13.0	45.2	92.6
Control (tap water)	25.5	54.9	79.3	121.8
CD (p=0.05)	1.02	2.31	8.52	12.18

ii) *In vitro* study on nematode penetration

Culture filtrate of actinobacteria (V5) was tested to understand its effect on juvenile penetration (Fig.2). The results showed that the culture filtrate of actinobacteria V5 isolate had an inhibitory effect on nematode penetration (Table 4). The results showed a significant reduction juvenile penetration ($t_c > t_t$). Culture filtrate application reduced 33 per cent penetration compared to control. The same observation was reflected in the number of galls per plant as well as number of eggmasses per root system (Table 4). There was only less influence of actinobacteria on egg mass production which was 28.6 in control and 24.8 in treated plants respectively.

Table 4: Effect of actinobacteria (V5 isolate) on *M. incognita* penetration and development

Treatments	No. of juveniles penetrated / plant	No. of galls / plant	No. of egg masses / root system
T1 – Control (sterile water 10ml /cup)	49.9	21.8	28.6
T2 - Actinobacteria culture filtrate (50 % @ 10ml / cup)	33.4	15.7	24.8
t_t	2.16	2.16	2.16
t_c	9.02	5.12	3.11

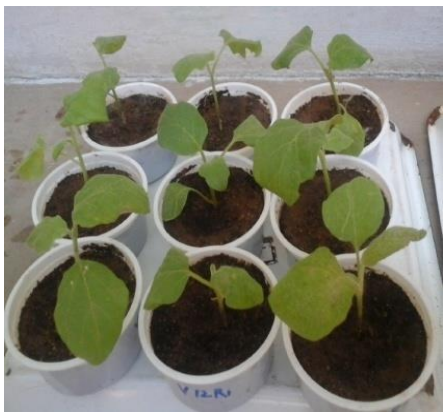


Fig 2: Experimental set of penetration study

iii) Pot culture experiment

A pot culture experiment was conducted to study the efficacy of native isolate of actinobacteria in comparison with other bioagents. The results revealed that actinobacteria (50% culture filtrate reduced soil and root nematode population (Table 5). Among different bioagents tested the egg parasitic fungus, *P. lilacinum* recorded with highest reduction in egg mass production (36.5 / 5g root) followed by actinobacteria (53.2 / 5g root). This results indicate that the actinobacteria is a potential bioagent. The dosage, concentration and time of application needs to standardized for effective management of root knot nematode in brinjal.

Table 5: Effect of different bioagents on *M. incognita* under Pot culture condition

Treatments	No. of nematodes / 250g soil	No. of galls / 5 g root	No. of egg masses / 5 g root
T1 – Actinobacteria (50% culture filtrate isolate V5)	324.7	68.2	53.4
T2 - *P. lilacinum* @ 10g / pot	227.3	11.5	36.5
T3 - *T. viride* @ 10g / pot	244.0	23.3	88.8
T4 - Carbofuran@ 3g / pot	300.7	74.5	101.7
T5 - Control	350.0	87.3	120.0
CD (p=0.05)	8.50	0.85	3.67

Discussion

The results of the above study revealed that the native isolates of bacteria and actinomycetes have an inhibitory effect on nematodes. Actinomycete isolate V5 was more effective than others. Similar results were obtained by [6] who reported that the native isolates of actinomycetes inhibited egg hatching and juvenile mortality under *in vitro* condition. They have identified the taxa as *Streptomyces* sp. They also found that culture filtrate resulted in 33.1% reduction in egg hatching and increased juvenile mortality rate to 82% compare to control. Nematicidal property of actinomycetes was tested under pot culture condition using *Streptomyces* sp. in tomato against *M. incognita* [7]. Current experiment result showed that the penetration and development was affected by actinomycetes soil application. This may be due to the toxins present or secreted by actinomycetes. This finding is supported by the works of [8] who tested the effect of the metabolites of actinomycetes on the movement of the root knot nematode, *M. incognita*. They have reported that the juveniles became inactive by all the isolates tested. They have also reported that the secondary metabolites were only nematostatic and not nematicidal. Actinomycete such as Streptomyces sp. releases avermectins which is a macrocyclic lactone. Avermectins are reported to block the nerves transmission and muscle cells by stimulating the release and binding of gamma-Aminobutyric acid at nerve endings [9]. These binding result in paralysis of the neuromuscular systems that adversely affect nematode hatching and movement in soil, subsequently reducing the extent of root invasion [10]. The observations of pot culture experiment showed that the egg parasitic fungus performed better than actinomycetes. *P. lilacinum* colonizes eggs of root knot nematode which results in lesser egg mass production were compared to other treatments.

Conclusion

Root knot nematode management in vegetable crops is a great challenge to farmers as well as scientists. Recent banning of nematicides urges scientists to evolve novel methods of nematode control. There is few commercial formulation containing secondary metabolites of actinomycetes. The current study indicates that the native isolate of actinomycetes have a positive remark in inhibiting root knot nematode hatching, penetration and development. The isolate was used as such and role of secondary metabolites were not tested. Hence, there is a scope of exploiting metabolites derived from this isolate also.

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