

Allelopathic potential of *Streptomyces lydicus* strain WYEC 108 and *Verticillium chlamydosporium* strain IIHR VC-3 to control root knot nematode (*Meloidogyne incognita*) in tomato

N.K. Dhillon*, S. Kaur¹, Anupam and H. S. Buttar
Department of Plant Pathology,
Punjab Agricultural University, Ludhiana-141004, India
E. Mail: narpinder@pau.edu

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ABSTRACT

Two bioformulation viz., *Streptomyces lydicus* strain WYEC 108 and *Verticillium chlamydosporium* strain IIHR VC-3 were evaluated against root knot nematode, (*Meloidogyne incognita*) infesting tomato crop. Both the bio formulations showed antinemic properties. *In vitro* studies revealed that the nematocidal potential of *Streptomyces lydicus* bioformulation was higher than *Verticillium chlamydosporium* formulation in decreasing the egg hatch count and increasing juvenile mortality. Field application of *Streptomyces lydicus* strain bioformulation at 560 g/ha and above significantly decreased root knot nematode population in soil and roots of tomato. The application of *Streptomyces* formulation increased the crop growth and yield. These studies revealed that *Streptomyces lydicus* bioformulation has the potential for use in ecofriendly management of root knot nematode in tomato under protected and open field conditions.

Keywords: *Allelopathic potential*, Bioformulation, egg hatch, juvenile mortality, *Meloidogyne incognita*, nematocidal, ovicidal, root knot nematode, soil, *Streptomyces lydicus*, tomato, *Verticillium chlamydosporium*,

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is most widely cultivated solanaceous crop as fresh vegetable and processing purpose. It is most important vegetable crop next to potato globally. Tomato is being grown under a wide variety of agro climatic zones ranging from the tropics to temperate regions (28). The sustainable production of the crop is affected by different biotic and abiotic stresses. Amongst the biotic stresses, plant parasitic nematodes of genus *Meloidogyne* are becoming important pathogens causing huge losses worldwide (21,35). They are prevalent in all temperate and tropical areas (5). Tomato crop is reported to suffer yield loss up to 22 - 70 % in tropical and sub tropical regions alone due to root-knot nematode, *Meloidogyne incognita* (19). The management of these nematodes is thus necessary for sustaining productions. Vegetables being directly consumable, use of the pesticides is hazardous to health. The indiscriminate application of pesticides for the management of plant diseases and pests has been discouraged widely (26). Further, appearance of new strains of pathogens resistant to chemicals and implementation of new laws concerning food quality and environmental conservation has led to the need for development of alternative disease management strategies in plant protection (11). Nowadays, allelopathy has become a viable alternative in natural crop protection (17). Allelopathy involves the release of chemical substance or antibiotic by a microorganisms, into a medium or an environment which inhibit the growth to another organism.

*Correspondence Authors, ¹Department of Vegetable Science

Biological control agents are gaining importance because of their non-hazardous nature as well as due to the synthesis of natural toxins or allelochemicals which inhibit different plant pathogens (20). These allelochemicals have a great potential to be used as natural pesticides against a number of plant pathogens (1,15). Of these microbial antagonists, actinobacteria (Gram positive bacteria) have been reported as potential biocontrol agents against various plant pathogens (fungi and bacteria) and pests (insects, mites).

In the recent decades, there is an increasing trend for the use of microorganisms as biocontrol agents in agriculture as main tools for controlling different crop pests and enhancing crop yields. Thus, the search for new potential microbial antagonists and their metabolites has gained momentum as safe and ecofriendly alternative to the agrochemicals. Various fungal or bacterial organisms which exhibit antagonism to nematodes are increasingly being explored for their potential in management of nematodes. Efforts have been made in the past years to identify microbial groups which can limit the nematode densities in soil and these are categorized into egg-parasitic fungi, nematode-trapping fungi, filamentous fungi, antagonistic microbial organisms and polyphagous predatory nematodes (24,25). The facultative egg parasitic fungus, *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (*Paecilomyces lilacinus*) are effective against *Meloidogyne* spp. and many other plant parasitic nematodes in various crops (9,29). Biocontrol agents viz., *Trichoderma longibrachiatum*, *Trichoderma asperellum*, *Bacillus subtilis*, *Bacillus cereus* and *Pseudomonas fluorescens* were proved to possess allelopathic potential against pathogens associated with tuber rot of *Dioscorea cayenensis* (10). *Streptomyces* spp. has also been reported to suppress the root-lesion nematode (*Pratylenchus penetrans*) in alfalfa (32). In this context, the efficacies of the strains of commercial available bioformulations need to be evaluated against root knot nematode, *M. incognita*, in view of the farmers need for sustaining productions. In the present studies, two bioformulations viz., *Streptomyces* and *Verticillium* were evaluated *in vitro* and *in vivo* for their efficacy against *M. incognita*. *Streptomyces* are the gram-positive filamentous Actinomycetes predominantly found in soil and decaying vegetation, which produce a wide array of biologically active compounds including antibiotics, hydrolytic enzymes (proteases, nucleases, lipases and a variety of enzymes hydrolyzing poly-saccharides), enzyme inhibitors and are resistant to desiccation and nutrient stress. All these characteristics make them attractive candidates for biological control agents against soil-borne pathogens (33). *Verticillium chlamydosporium* on the other hand is a widely prevalent rhizosporic fungus which parasitizes eggs and females of root knot nematodes (34).

MATERIALS AND METHODS

Studies were conducted *in vitro* and *in vivo* to explore the allelopathic effects of *Streptomyces lydicus* strain WYEC 108 with CFU count @ 1×10^7 /gm and *Verticillium chlamydosporium* strain IIHR VC-3 with CFU count @ 2×10^6 /gm against *M. incognita*. and are discussed below.

Ovicidal and larvicidal efficacy of *Streptomyces lydicus* strain WYEC 108 and *Verticillium chlamyosporium* strain IIHR VC-3 against *M. incognita*

The commercial bio formulation was taken as the standard stock solution (S) with 1×10^7 CFU per gram. Further dilutions of this stock solution were made to get concentrations of 0.25 %, 0.5 %, 1.0 %, 1.5 % and 2.0 % concentrations. These were evaluated along with carbofuran (250 μ /ml) which served as nematicide control for their ovicidal and larvicidal effect. For ovicidal studies, uniform sized egg masses of *M. incognita* were extracted from pure culture maintained on tomato in the pot house of Department of Plant Pathology.

Ten egg masses of approximately same size (sterilized with 0.5 % sodium hypochlorite) were placed in Petri dish containing 10 ml of each concentration. These were regularly observed under stereo binocular microscope after 24, 48, 96 and 144 h for number of second stage juveniles hatched. For larvicidal studies, hundred specimens of freshly hatched second stage juveniles of *M. incognita* concentrated in 10 ml water were transferred to ten ml double strength concentrations of each treatment to make the required concentration of 20 ml volume (0.25 %, 0.5 %, 1.0 %, 1.5 % and 2.0 %) in Petri dishes. Carbofuran was taken as taken as the nematicidal check. The nematodes after duration exposed were transferred to water for their revival studies. Observations were taken at regular intervals of after 24, 48, 96 and 144 h on the number of dead nematodes and per cent juvenile mortality was calculated as under:

$$\frac{T_D}{T_C} \times 100$$

Where, T_D - Total number of nematodes dead in treatment and T_C : total number of nematodes in control

Efficacy of *Streptomyces lydicus* strain WYEC 108 against *M. incognita* in tomato under pot and field conditions

Of the two formulations evaluated *Streptomyces lydicus* strain WYEC 108 was found to be more effective than *Verticillium chlamyosporium* strain IIHR VC-3. Keeping this in view, *Streptomyces* formulation was further evaluated for one season in pots and for two seasons under field conditions against root knot nematode. For pot trials, nursery of tomato variety cv Punjab Ratta was raised in trays containing mixture of cocopeat, perlite and vermiculite. Healthy seedlings of tomato at 3-4 leaf stage were transplanted in 15 cms diameter pots containing 1 kg root knot nematode infested soil/pot (with initial population of 263.3 of $J_2/250$ cc soil). The formulation was applied @140, 280, 560 and 840 g/ha respectively in two applications, first at the time of transplanting and second at 30 days after transplanting along with a treated check (Carbofuran @ 2 kg *a.i./ha*) and control (no treatment). Each treatment was replicated thrice. Observations on soil nematode population/250 cc soil, root galling index (RGI) and plant growth parameters were recorded on seventy days old crop. Soil nematode population estimation was done after washing soil samples using Modified Cobb's decanting and sieving method (8) and RGI was taken using 0-10 rating scale (4).

For testing the efficacy of *Streptomyces* formulation under field conditions, raised beds of 1m x 3m size were prepared in root knot nematode infested field maintained in the Department of Plant Pathology, PAU, Ludhiana. The seeds of tomato variety cv Punjab Ratta

were sown in plug trays filled with mixture of cocopeat, perlite and vermiculite to raise seedlings. The seedlings at 3-4 leaf stage were transplanted to the field. During first year transplanting was done on 24th October and in second year on 30th October. The bio formulation was applied at four different doses viz., @140, 280, 560 and 840 g/ha in two applications, first at the time of transplanting and second at 30 days after transplanting along with a treated check (Carbofuran @ 2 kg a.i./ha) and control (no treatment). Each treatment was replicated thrice. Observations were recorded on nematode population/250 cc soil and root galling index (1-10 scale) and crop yield.

RESULTS AND DISCUSSION

Ovicidal and larvicidal efficacy of *Streptomyces lydicus* strain WYEC 108 and *Verticillium chlamydosporium* strain IIHR VC-3 against *M. incognita*

The analysis of variance revealed significant effect of *Streptomyces lydicus* strain WYEC 108 and *Verticillium chlamydosporium* strain IIHR VC-3 on hatching and mortality of *M. incognita* juveniles. Studies conducted *in vitro* revealed that both the bio formulations reduced egg hatching of root knot nematode, *M. incognita* as compared to untreated control. The inhibition of egg hatch was observed in dose dependent manner which increased from 31 to 99%. Maximum percent egg hatch inhibition in *Streptomyces lydicus* strain WYEC 108 application was recorded at 2 per cent concentration (99.91 %) followed by 1.5 per cent concentration (90.25 %) and minimum egg hatch inhibition was observed at 0.25 % per cent concentration (Fig. 1a). Higher concentration of *Streptomyces* exhibited nematicidal efficacy than carbofuran. Comparatively, lower egg hatch inhibition was observed in *Verticillium chlamydosporium* strain IIHR VC-3, viz., 58.17 % at 2 per cent concentration and 52.72 % inhibition at 1.5 per cent concentration (Fig. 1b). The results indicated greater efficacy of *Streptomyces lydicus* strain WYEC 108 bio formulation than *Verticillium chlamydosporium* strain IIHR VC-3 formulation in suppressing hatching of eggs of *M. incognita* (Fig. 1e). Further, it was found that the egg hatch count was duration dependent and it increased in all the treatments with time (Fig. 2a). Total number of juveniles hatched after 144 hrs were minimum in *Streptomyces lydicus* strain WYEC 108 followed by *Verticillium chlamydosporium* strain IIHR VC- and maximum in case of control treatment. The lethal concentration for 50 % egg hatch inhibition (LD 50) for *Streptomyces lydicus* strain WYEC 108 was 0.50 % whereas, for *Verticillium chlamydosporium* strain IIHR VC-3 it was observed at higher concentration of 1.5% (Fig. 3).

A perusal of Fig.1 c & d showed that both the bio formulations affected *M. incognita* juvenile mortality to different extent at different concentrations. *Streptomyces lydicus* strain WYEC 108 formulation showed maximum juvenile mortality at 2 per cent concentration (67.99 %) followed by 1.5 per cent concentration (56.74 %) and minimum at 0.25 per cent concentration. Similarly, *Verticillium chlamydosporium* strain IIHR VC-3 formulation also showed maximum juvenile mortality (52.91 %) at 2 per cent concentration and minimum (15.75 %) at 0.25 per cent concentration. Thus, the nematode juvenile mortality was also observed to be concentration dependent giving minimum mortality at 0.25 % concentration and maximum mortality at 2.0 % concentrations for both the bio formulations. However, significantly higher juvenile mortality was observed in

Streptomyces lydicus strain WYEC 108 formulation as compared to *Verticillium chlamyosporium* strain IIHR VC-3 formulation and control (Fig 1f). The juvenile mortality was also found to be exposure time dependent. The juvenile mortality increased in all the treatments with increase in exposure time showing maximum mortality after 144 h (Fig. 2b).

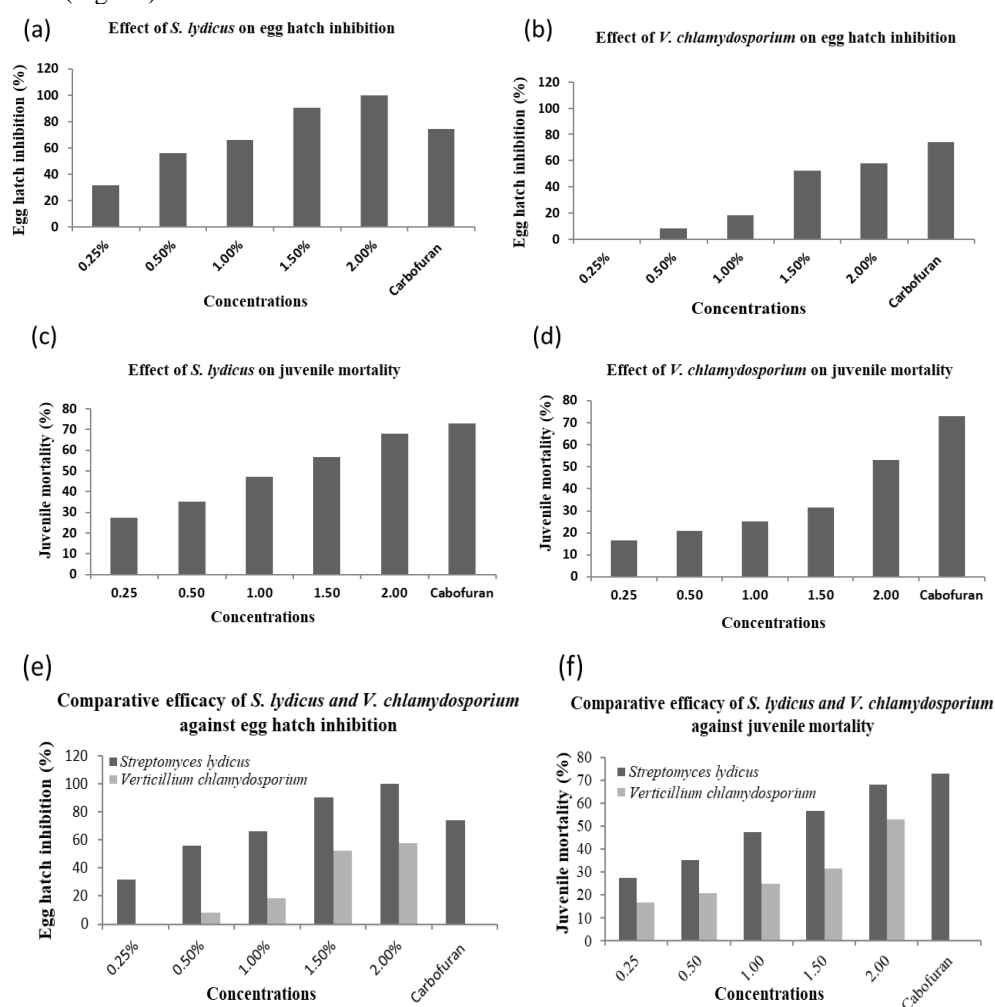


Figure 1. Efficacy of bio formulations on per cent egg inhibition and juvenile mortality of root knot nematode at different concentrations: (a) Effect of *Streptomyces lydicus* on per cent egg hatch inhibition; (b) Effect of *Verticillium chlamyosporium* on per cent egg hatch inhibition; (c) Effect of *Streptomyces lydicus* on per cent juvenile mortality; (d) Effect of *Verticillium chlamyosporium* on per cent juvenile mortality; (e) Comparative efficacy of *Streptomyces lydicus* and *Verticillium chlamyosporium* on per cent egg hatch inhibition; (f) on per cent juvenile mortality.

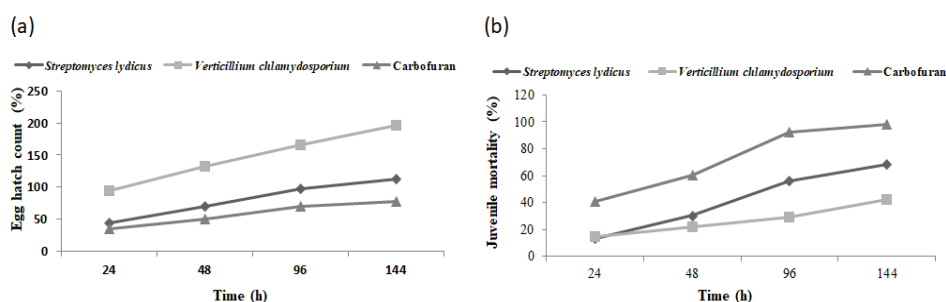


Figure 2. Effects of *Streptomyces lydicus* and *Verticillium chlamydosporium* bio formulations on per cent egg hatching (a) and per cent juvenile mortality (b) of root knot nematode at different times.

The inhibitory effects of *Streptomyces lydicus* strain WYEC 108 has been related to degradation of biopolymers (lignocellulose, starch and chitin) that possess larvicidal properties (13). *Streptomyces sp.*, CMU-MH021, was reported to produce secondary metabolites that cause 82 % J2s mortality of *M. incognita* in (31). Also *Streptomyces rubrogriseus* HDZ-9-47, isolated from eggs of *Meloidogyne* spp. parasitized eggs of *M. incognita* and caused 97.0 % mortality of second-stage juveniles (J2s) of *M. incognita* and also more than 50 % egg hatch inhibition (21). Actinomycetes, *Streptomyces lydicus* have been reported to act as nematode antagonists by producing certain allelopathic chemicals such as antibiotics, hydrolyzing *in vitro* enzymes, secondary metabolites and siderophores (7,13). *Verticillium chlamydosporium* infects nematode eggs and sedentary females by hyphae produced on actively growing mycelium (23) thus reducing the hatch count of the root knot nematode. However, its efficacy as a potential bio control agent is reported to vary among the isolates. *Streptomyces rubrogriseus* HDZ-9-47, isolated from eggs of *Meloidogyne* spp. parasitized eggs of *M. incognita* and has been reported to cause 97.0 % mortality of second-stage juveniles (J2s) of *M. incognita* with more than 50 % egg hatch inhibition (22)

Efficacy of *Streptomyces lydicus* strain WYEC 108 against root knot nematode in tomato

Studies conducted *in vitro* revealed that *Streptomyces lydicus* strain WYEC 108 formulation was comparatively more effective than *Verticillium chlamydosporium* strain IIHR VC-3 formulation in suppressing the nematode egg hatching and larval mortality. In view of this, further studies in pot house and field were conducted on *Streptomyces lydicus* strain WYEC 108 formulation for their allelopathic potential against root knot nematode.

Results of pot experiment recorded at 70 days old crop revealed that application of *Streptomyces lydicus* strain WYEC 108 bioformulation @ 280, 560 and 840 g/ha significantly reduced nematode infestation in soil as well as in roots of tomato. Maximum reduction in soil nematode population was observed when bio formulation was applied @ 840 g/ha (42.02 %) closely followed by treatment with application of the bio formulation @ 560 g/ha (33.89 %). However, these two doses were statistically at par with each other in reducing the nematode infestation in soil. RGI in roots was also observed to be least, when the formulation was applied at rate of 840 g/ha (37.59%), followed by application at

the rate of 560 g/ha (30.82%). The decrease in nematode population at higher dose of 560 and above was statistically at par with treated control (Carbofuran @ 2 kg a.i./ha). Minimum reduction of nematode infestation in soil and root was observed in treatment with application of *Streptomyces lydicus* strain WYEC 108 bioformulation @140 g/ha. Plant growth parameters viz., plant height and plant weight was observed to increase with the application of *Streptomyces* bio formulation.

Table 1. Effects of *Streptomyces lydicus* formulation on root knot nematode infestation and plant growth parameters in pots

Treatment	Soil Population /250 cc soil	Per cent decrease over control	Root Gall Index (0-10) scale	Per cent decrease over control	Plant height (cm)	Plant weight (g)
<i>Streptomyces lydicus</i> @ 140 g/ha	568.30 (23.85)	9.16	7.90	0.00	36.19	5.86
<i>Streptomyces lydicus</i> @ 280 g/ha	514.78 (22.71)	17.72	7.17	9.30	42.57	7.41
<i>Streptomyces lydicus</i> @ 560 g/ha	413.60 (20.35)	33.89	5.47	30.82	48.33	9.24
<i>Streptomyces lydicus</i> @ 840 g/ha	362.76 (19.07)	42.02	4.93	37.59	47.66	9.21
Carbofuran @ 2kg a.i/ha	362.28 (19.05)	42.09	4.80	39.24	48.12	8.87
Untreated Control	625.63 (25.03)	-	7.90	-	35.66	6.56
CD (0.05%)	0.91		1.41		2.46	1.08

*Figures within brackets are square root transformed values

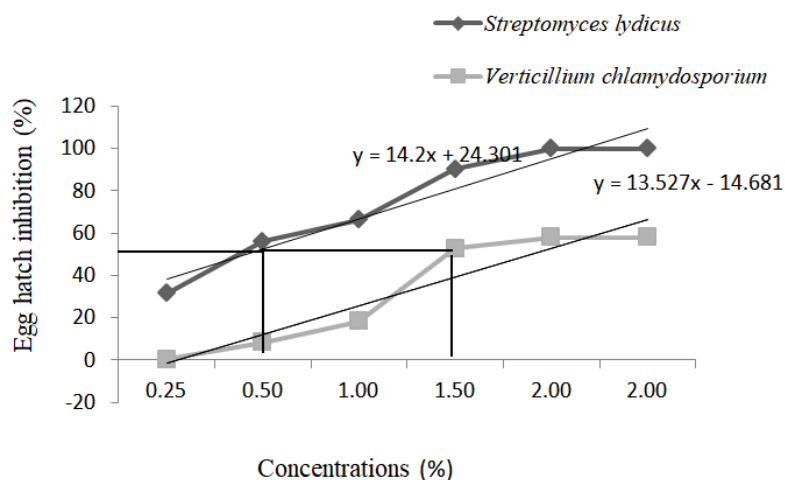


Figure 3. Graph showing LD 50 value for *Streptomyces lydicus* and *Verticillium chlamydosporium* bioformulations against root knot nematode, *M. incognita* egg hatching.

Maximum increase in plant height and weight was observed with application of *Streptomyces lydicus* strain WYEC 108 formulation @560 g/ha and which was statistically at par with application of the formulation @840 g/ha (Table 1; Fig. 4).

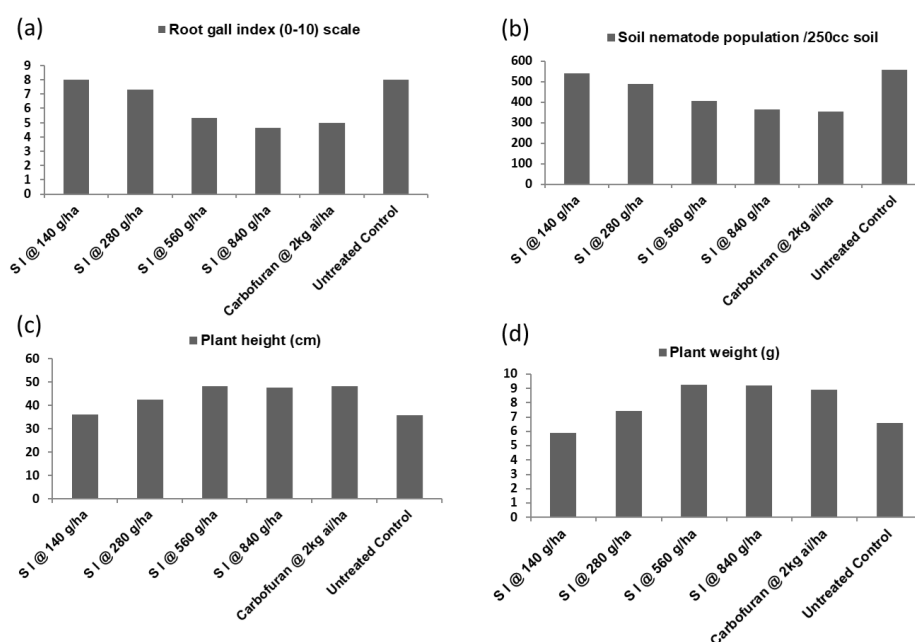


Figure 4. Effects of *Streptomyces lydicus* bio formulation on (a) root gall index; (b) soil nematode population; (c) plant height; (d) plant weight of tomato grown in pots.

Trials conducted in the root knot nematode infested field for two seasons showed that higher dosages of *Streptomyces lydicus* strain WYEC 108 formulation were effective in managing root knot nematode in tomato. Soil nematode population was observed to be least in application of *Streptomyces* @ 840 indicating 38 % decrease in nematode population as compared to control followed by application of bioformulation @ 560g (33 %). However, the decrease in nematode population in both these treatments was observed to be significantly at par with each other. The decrease in nematode population in carbofuran treated plots was observed to be statistically at par with *Streptomyces* treated plots @ 560 and above. Application of *Streptomyces* @ 140g/ha decreased soil nematode population by 4.21% indicating its inefficacy in decreasing root knot nematode population at this dosage. Maximum decrease in root galling index was also observed in higher dosages of 560 and 840gm/ha of *Streptomyces* formulation (44.5 - 48.9 %) and it also increased marketable yield (Table 2).

Table 2. Effect of *Streptomyces lydicus* bioformulation on soil nematode population, root gall index and yield of tomato grown in infested field (Pooled Data 2017 and 2018)

Treatment	Soil nematode population / 250 cc soil	Per cent decrease over Control	Root Gall Index (0-10) scale	Per cent decrease over Control	Yield kg/ plot
<i>Streptomyces lydicus</i> @ 140 g/ha	538.13 (23.21)	4.21	6.2	3.42	11.30
<i>Streptomyces lydicus</i> @ 280 g/ha	466.63 (21.61)	9.63	5.8	9.65	12.50
<i>Streptomyces lydicus</i> @ 560 g/ha	343.63 (18.54)	33.45	3.56	44.5	13.30
<i>Streptomyces lydicus</i> @ 840 g/ha	318.69 (17.86)	38.28	3.28	48.9	14.30
Carbofuran @ 2kg ai/ha	329.95 (18.19)	36.10	3.38	47.3	14.0
Untreated Control	516.37 (22.75)	-	6.42	-	14.50
CD (0.05%)	1.31		0.65		1.46

**Figures within brackets are square root transformed values

The establishment in the rhizosphere is key to success of biocontrol agents using antagonistic fungi. Prior to parasitism and predation, introduced nematophagous fungi must be able to grow and colonize the soil or rhizosphere, which requires energy (27). The biocontrol efficacy of the *Streptomyces* formulation could be attributed to its ability of root colonization alongwith production of active nematicidal metabolites in the soil which may have allelopathic properties that restrict the nematode population. The nematicidal compounds released perhaps provided sustainable protection to crops to attack by nematodes (2). The trials conducted in pot and field revealed that *Streptomyces* formulation improved the growth of tomato plants. The improved plant growth parameters indicate the positive effect of the metabolites. The infections caused by nematodes predispose them to penetration of *Streptomyces* into plant tissues through wounded root and hence the bioactive metabolites directly secreted in root tissues had an impact on the growth of tomato plants (30). Furthermore, even in the absence of nematode stress, soil drenching with culture supernatant/cells significantly promoted plant growth in terms of shoot and root lengths, and fresh and dry weights (14). The enhancement in different plant growth parameters by *Streptomyces* spp. has been reported with ability of the strain for IAA production, ammonia production, ACC deaminase activity, root colonization and nitrogen fixation (36). The nematicidal properties of are also due to production of ionophores, enzyme inhibitors or avermectins and hydrolyzing enzymes viz., chitinases, proteases, nucleases, lipase and polysaccharides (3,6). The extracellular enzymes enable them to degrade various biopolymers in soil and these enzymes also exhibit bioactive properties against living organisms (16,18) which become a cause for decreasing nematode infestations.

CONCLUSIONS

These findings indicated that both *Streptomyces lydicus* strain WYEC 108 and *Verticillium chlamydosporium* strain IIHR VC-3 bioformulations were antinematic against root knot nematode, *M. incognita*. Allelopathic potential of *Streptomyces lydicus* strain WYEC 108 *in vitro* was comparatively higher than *Verticillium chlamydosporium* strain IIHR VC-3. Application of *Streptomyces lydicus* strain WYEC 108 bioformulation (CFU 1×10^7) @ 560 g/ha and above significantly decreased root knot nematode infestation in soil and roots of tomato and increased growth parameters. These have the potential to control nematode infestations and to reduce its harmful effects on crop production. Therefore, the use of *Streptomyces* strain and its metabolites can be used as a safer eco-compatible approach and an effective fertilizer to manage root knot nematode in tomato under protected and open field conditions. Trials need to be conducted further for enhancing its properties by integrating it with amendments or other applications.

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