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RESEARCH

Bare-root-dip treatment of tomato seedlings with neem and *Bacillus thuringiensis* for the management of *Meloidogyne incognita*

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ABSTRACT

Root-knot nematode, *Meloidogyne incognita* is one of the major limiting factors affecting plant growth and yield. Currently, synthetic pesticides are principle means used to control the nematodes but organic amendments and biocontrol agents may provide a safer alternatives. A study was conducted to evaluate the nematicidal potential of neem and *Bacillus thuringiensis* in controlling *M. incognita*. 5% neem leaf extract @ 10ml/seedling, 10% neem seed extract @ 10ml/seedling, 1.1X 10^9 CFU/ml Bt formulations alone @ 10ml/seedling and neem+Bt in combination in the ratio1:1 and 2.5% carbofuradan 3-G @ 10ml/seedling to control *M. incognita* infesting tomato plants. Bare-root-dip treatment with NSE was effective and caused 97.3%, and 120.3% significant increase ($p<0.05$) in total plant length and total plant weight respectively as compared to the inoculated control. The present study indicated that carbofuradan 3-G and WCS+NSE used as bare-root-dip treatment caused significant reduction in number of galls/root system by 75.5% and 42.1% as compared to the inoculated control. The bare-root-dip treatment with carbofuradan 3-G, WCS+NLE, CPR+NLE and CPR were effective and caused reduction in number of egg masses/gall by 70%, 49%, 48%, 41% respectively as compared to the inoculated control. The efficacy of carbofuradan 3-G, WCS+NLE, CPR, WCS+NSE as bare-root-dip treatment in reducing number of J2/1g root i.e. 86.8%, 68.9%, 68.4% and 67.3% respectively as compared to the inoculated control. Bare-root-dip treatment with WCS+NSE was effective and caused 63.6% reduction in number of males/250g soil as compared to the inoculated control.

Key words: Bare-root-dip treatment, biocontrol, *Meloidogyne incognita,* egg masses, *Bacillus thuringiensis*, carbofuradan 3-G

1. INTRODUCTION

Tomato (*Lycopersicum esculentum* mill) is one of the most widely grown vegetable in the world ranking second in importance to potato in many countries. It belongs to family Solanaceae. In India tomato, cultivated in about 80, 000 hectares of land. It is essential for balanced diet and maintenance of good health (Mastol *et al.,* 2006).

Plant parasitic nematodes are important pests of many cultivated vegetables. The *Meloidogyne* genus belongs to a group of root-knot nematodes (RKN) and is represented by over 90 species that have been described so far (Moens *et al*., 2009). These are ubiquitous soil organisms with a wide host range. It is ranked among the most damaging plant pathogen (Sasser *et al.,* 1984). Infested plants shows the symptoms of stunting, yellowing, aberrant development of root system characterized by the formation of typical galls, a general unthrifty appearance and limited fruit production, estimated yield loses ranging from 28% to 68% (Adesiyan *et al.,* 1990, Williamson *et al.,* 1996). Therefore, there is great need to control the root-knot nematode, *Meloidogyne incognita* infesting tomato plants.

The success of pesticides in the middle of the 20th century enabled control of many harmful organisms. The pesticides introduced new environmental conditions to which plant pathogens had to adapt, frequently by becoming resistant. Due to the adverse effects of pesticides on the environment and human health, this investigation aimed to evaluate the performance of organic amendment-neem and biological control agent- *Bacillus thuringiensis* in comparison with synthetic nematicides to control *M. incognita* in tomato.

The present study was carried out to determine the impact of neem alone, Bt alone and neem + Bt combinations on the *Meloidogyne incognita.*

2. MATERIALS AND METHODS

2.1. Preparation of Bt and neem formulations

2.1.1. Accession of *Bacillus Thuringiensis*

Bacillus thuringiensis strain MTCC CODE 1953 was accessed from Institute of Microbial Technology IMTECH, Sector-39 A, Chandigarh-160036, India.

2.1.2. Collection of Neem Leaves and Neem Seed Powder

During the period of four years i.e. 2009-2012, mature leaves and seeds of neem (*Azadirachta indica)* (Juss, 1830*)* were collected from Punjabi University campus. Leaves were shade dried and were grinded in electric grinder. Oil was extracted from the neem seeds and rest of neem khali (seed coat) was grinded to obtain the powder form.

2.1.3. Accession Of Carbofuradan 3-G

Carbofuradan 3-G was accessed from Bharat Seeds, Luv Kush market, Patiala and was used as chemical check.

2.1.4. Revival and Maintenance of Bt Culture

20ml nutrient broth in a flask was inoculated with lyophilized culture of Bt and incubated at 30°C for 24 h. Bt culture was maintained on agar plates, for this 20 ml of nutrient broth inoculated with a loopful of Bt was thoroughly mixed and incubated at 30°C for 24 h followed by streaking on agar plates in quadrant manner with the help of inoculating needle. Streaked plates were kept in inverted position at 30°C for 24 h to obtain Bt colonies.

2.1.5. Preparation of Four Bt Formulations

a. *Whole Cell Suspension (WCS)*
   20ml of nutrient broth was taken and inoculated with one colony of Bt. Kept overnight at 30°C (flask-A). 20 ml fresh nutrient broth was inoculated with 0.2ml of Bt from flask-A and incubated for 6 h to obtain Bt cell suspension (1.1×10^9 CFU/ml) (Mohammed, 2008).
b. *Cell Free Supernatant (CFS)*
   20 ml of nutrient broth was taken and inoculated with one colony of Bt incubated overnight at 30°C. (Flask-A) 20ml fresh nutrient broth was inoculated with 0.2ml of Bt from flask-A and incubated for 24 h to obtain Bt cell suspension (1.1×10^9CFU/ml). The Bt cell suspension was centrifuged at 3000rpm for 15 minutes and washed 3 times with 0.85% NaCl to obtain cell free supernatant (Mohammed, 2008).
c. *Cell pelleted Residues (CPR)*
   20 ml of nutrient broth was inoculated with one colony of Bt, incubated overnight at 30°C (Flask-A). 20ml fresh nutrient broth was inoculated with 0.2ml of Bt from flask-A and was incubated for 24 h to obtain Bt cell suspension (1.1×10^9CFU/ml). Bt cell suspension was centrifuged at 3000rpm for 15 minutes and washed 3 times with 0.85% NaCl to obtain cell pelleted residue Mohammed, 2008).
d. *Spore/Crystal Proteins (SCP)*
   20ml of nutrient broth was inoculated with one colony of Bt and incubated overnight at 30°C (Flask-A). 20ml suspension of flask-A was centrifuged at 3000rpm and supernatant was discarded and 10 ml distilled water was added into the pellet in 100ml beaker. Sonication was perfomed in 100ml beaker placed in 500ml beaker containing crushed ice. Sonication was done for 4 cycles of 30 seconds each at 15 amplitude micron (Mohammed *et al*., 2008) to obtain Spore/Crystal Proteins.

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2.1.6. Preparation of Aqueous Neem Leaf Extract and Neem Seed Extract

25g of neem leaf powder and neem seed powder was blended in electric blender in 250ml distilled water for 15 minutes, kept in water bath for 8h at 60°C, autoclaved at 15Ib pressure at 121°C, allowed to cool and filtered through the muslin cloth. Filtrate was considered as standard solution (100%) (Akhtar and Mahmood, 1994).

2.2. Culture of *M. Incognita* Maintained on Tomato Plants

The root-knot nematode, *M. incognita* was cultured on tomato (*Lycopersicon esculentum*) cv Pusa Ruby in earthen pots (15cm diameter) under green house conditions (22-28^{o}C), Punjabi University, Patiala. The egg masses collected from galled roots were shaken in 1% sodium chloride solution for 2 min in electric blender and then washed several times in distilled water and were allowed to hatch. J2 were collected in a petridish for 24h for inoculation in potted tomato plants.

2.3. Greenhouse Experiments

The effect of standard solution (100%) of aqueous neem leaf extract, neem seed extract, Bt formulations (1.1X 10^9 CFU/ml) such as whole cell suspension, cell free supernatant, cell pelleted residues and spore/crystal proteins and neem + Bt formulations on *M. incognita* and tomato plants was studied in greenhouse (22-28^{o}C) using earthen pots (15 cm diam). In addition, carbofuradan 3-G (chemical check) and green manure (organic amendment) were selected for comparison. All these formulations were used as seed dressing treatment. Soil for experimentation was obtained from non-cultivated dry localities and sun dried for 15 days. Each pot was filled with 6kg soil (Siddiqui, 2000).

2.4. Bare-Root-Dip Treatment Experiment Design

Seeds of tomato (*Lycopersicon esculentum* cv. Pusa Ruby) after surface sterilization in 1% soduim hypochlorite solution (NaOCl) were washed thoroughly under running tap water and allowed to dry under a laminar flow hood. The seeds were sown and 2 week old seedlings were transplanted in 15cm earthen pots containing 6 kg nematode free soil. At the time of transplantation, roots were treated by dipping for 15 min. in 5% neem leaf extract @ 10ml/seedling, 10% neem seed extract @ 10ml/seedling, 1.1X 10^9 CFU/ml Bt formulations alone @ 10ml/seedling and neem+Bt in combination in the ratio1:1 and 2.5% carbofuradan 3-G @ 10ml/seedling. After one week of transplantion of bare-root-dip treated seedling, the base of roots in each pot was inoculated with 3600J2 of *M. incognita* and pots were settled on greenhouse bench. Plants with nematode inoculum served as inoculated control (control-1) and without nematode inoculum as uninoculated control(control-2). Observations on total plant length, total plant weight and nematode control parameters such as number of galls, number of males/250g soil, number of females/gall, number of egg masses/gall, number of J2/1g root, number of J2/250g soil were recorded after 45 days of *M. inocognita* inoculation (Siddiqui, 2000). Treatments were replicated 5 times. Following treatment combinations were evaluated for bare-root-dip treatment:

i) Seedling+ neem leaf extract+ pathogen
ii) Seedling+ neem seed extract+ pathogen
iii) Seedling+ whole cell suspension+ pathogen
iv) Seedling+ cell free supernatant+ pathogen
v) Seedling+ cell pelleted residues+ pathogen
vi) Seedling+ spore/crystal proteins+ pathogen
vii) Seedling+ whole cell suspension+ neem leaf extract+ pathogen
viii) Seedling+ whole cell suspension+ neem seed extract+ pathogen
ix) Seedling+ cell free supernatant+ neem leaf extract+ pathogen
x) Seedling+ cell free supernatant+ neem seed extract+ pathogen
xi) Seedling+ cell pellted residues+ neem leaf extract+ pathogen
xii) Seedling+ cell pelleted residues+ neem seed extract+ pathogen
xiii) Seedling+ spore/crystal proteins+ neem leaf extract+ pathogen
xiv) Seedling+ spore/crystal proteins+ neem seed extract+ pathogen
xv) Seedling+ carbofuradan+ pathogen
xvi) Seedling+ pathogen (inoculated control-1)
xvii) Seedling (uninoculated control-2)

2.5. Statistical Analysis

Mean values for each experiment were calculated. Data recorded was analyzed statistically by using analysis of variance (ANOVA), Pearson correlation and means were compared with the Tuckey's Multiple Range test.

3. RESULTS AND DISCUSSION

Table 1 and Fig. 1 showed that bare-root-dip treatment with NSE, carbofuradan 3-G, WCS+NSE and CPR+NSE were effective and caused 97.3%, 80.6%, 88.2% and 80.09% significant increase ($p<0.05$) in total plant length i.e. 4.6cm, 4.5cm, 5.2cm and 2.8cm as compared to the inoculated control (29.04cm). Akhtar and Alam (1993a) also recorded maximum total plant length in nimin in bare-root-dip treated tomato plants and the increase was by 38 cm as compared to neem oil, castor oil, rocket-salad oil and mustard oil. Similar results by Tariq and Siddiqui (2005)

demonstrated that bare-root-dip treatment of tomato with neem cake + carbofuradan caused highest value of total plant length (50.8 cm) as compared to neem leaves, neem cake, carbofuradan and neem leaves + carbofuran. Carbofuradan 3-G was applied as bare-root-dip treatment caused 249.4% increase (19.5g) in total plant weight as compared to the inoculated control (5.6g). NSE used as organic amendment in bare-root-dip treatment caused 120.3% (12.3g) increase in total plant weight as compared to the inoculated control (Table 2 and Fig. 2). According to Akhtar and Alam (1993b) bare-root-dip treatment with standard concentration of nimin caused significantly maximum total plant weight of tomato i.e. 69.7g followed by S/2 and S/10 concentration i.e. 65 g and 62.7 g respectively over the inoculated control (76.7g).

The present study indicated that carbofuradan 3-G and WCS+NSE used as bare-root-dip treatment caused significant reduction in number of galls/root system by 75.5% and 42.1% as compared to the inoculated control. NSE was also effective and caused significant reduction by 33.3% as compared to the inoculated control (Table 3 and Fig. 3). Similar findings were by Akhtar and Alam (1993a) according to them the bare-root-dip treatment with standard concentration of nimin caused least number of galls as compared to neem oil, castor oil, rocket-salad oil and mustard oil. Saravanapriya and Sivakumar (2005) demonstrated that bare-root dip treatment with aak leaf extract caused lowest gall index of 1.7 followed by leaf extract of neem (2.4), marigold (2.9), seed extract of betel nut (3.4) and watermelon (3.5) whereas in control it was 5.0. Javed *et al.* (2007) recorded that bare-root-dip treatment with neem cake and aza (refined neem product) was more effective and gave minimum number of galls in tomato. Sahebani and Hadavi (2008) recorded that bare-root-dip treatment of tomato plants treated with filamentous fungi, *Trichoderma harzianum* caused minimum number of galls/plant at concentration 10^7, 10^8 and 10^9 spore/ml over the control.

The bare-root-dip treatment with carbofuradan 3-G, WCS+NLE, CPR+NLE and CPR were effective and caused reduction in number of egg masses/gall by 70%, 49%, 48%, 41% respectively as compared to the inoculated control. However, bare-root-dip treatment with SCP was ineffective and numerous egg masses/gall (23%) were recorded as compared to the inoculated control (Table 4 and Fig. 4). Khan *et al.* (2011) reported that bare-root-dip treatment of tomato plant with fruit extract of chilli caused maximum reduction in egg masses by 11% as compared to the control. Sahebani and Hadavi (2008) observed that filamentous fungus, *Trichoderma harzianum* caused significant reduction in egg mass/tomato plant at 10^7, 10^8 and 10^9 spores/ml over the control. The present study indicated that bare-root-dip treatment with carbofuradan 3-G and WCS+NLE caused 72.3% and 56% reduction in number of females/gall as compared to the inoculated control. However, bare-root-dip treatment with SCP was ineffective and caused increase in the number of females/gall by 2% as compared to the inoculated control (Table 5 and Fig. 5). Khan *et al.* (2011) reported that bare-root-dip treatment of tomato plant with fruit extract of chilli caused maximum reduction in mature females i.e. 10.6% as compared to the control. In the present study, NLE used as bare-root-dip treatment caused 31.3% reduction in number of females/gall as compared to the inoculated control. Javed *et al.* (2007) also reported that least number of females/root system i.e. 38.9% in aqueous neem leaf extract used as bare-root-dip treatment as compared to the inoculated control.

The efficacy of carbofuradan 3-G, WCS+NLE, CPR, WCS+NSE as bare-root-dip treatment in reducing number of J2/1g root i.e. 86.8%, 68.9%, 68.4% and 67.3% respectively as compared to the inoculated control (Table 6 and Fig. 6). Saravanapriya and Sivakumar (2005) demonstrated that bare-root-dip treatment with leaf extract of aak resulted in highest population reduction (87.3%) followed by neem (84.7%), marigold (72.6%), seed extract of betel nut (57.5%) and water melon (49.1%) than control. Akhtar and Alam (1993a) recorded that nimin significantly reduced the number of nematodes/250g soil on tomato plants followed by neem oil, castor oil, rocket-salad oil and mustard oil. Moreover, the potential of treatments was comparatively more in higher concentrations and it decreased with decreasing concentration. Sivakumar and Gunasekaran (2011) reported that in tomato the neem oil formulation N060EC(c) used as seedling bare-root-dip resulted in lowest number of nematodes in soil over the control. In the present study bare-root-dip treatment with carbofuradan 3-G, CPR, NLE, WCS+NLE, WCS+NSE and CPR+NLE were effective and caused reduction by 100%, 67.6%, 64%, 63.3%, 62.9% and 61.1% in number of J2/250g soil respectively as compared to the inoculated control (Table 7 and Fig. 7). Bare-root-dip treatment with WCS+NSE was effective and caused 63.6% reduction in number of males/250g soil as compared to the inoculated control (23.1) (Table 8 and Fig. 8).

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Table 1

Showing effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem and Bt on percentage increase/decrease in the total length (in cm) of the tomato plant infested with *M. incognita* as compared to infected (C-1) and normal plants (C-2)

Treatments	Total plant length (cm)		
	Mean	Reduction %age	Increase %age
Neem leaf extract	42.98± 3.2	26.7	47.7
Neem seed extract	52.45± 2.4	24.7	80.61
Whole cell suspension	33.81±4.0	51.4	16.42
Cell free supernatant	33.74± 3.0	51.5	16.18
Cell pelleted residues	30.40± 2.9	56.3	4.68
Spore/ crystal proteins	32.03± 2.8	53.9	10.29
Whole cell suspension + neem leaf extract	42.68± 1.8	38.7	46.9
Cell free supernatant + neem leaf extract	36.27± 2.9	47.9	24.8
Cell pelleted residues + neem leaf extract	37.51± 2.4	46.1	29.13
Spore/crystal proteins + neem leaf extract	35.15± 2.4	49.5	20.8
Whole cell suspension + neem seed extract	54.675± 3.6	20.6	88.2
Cell free supernatant + neem seed extract	42.62± 2.1	38.7	46.7
Cell pelleted residues + neem seed extract	52.38± 3.2	24.8	80.09
Spore/crystal proteins + neem seed extract	40.04± 3.9	41.9	37.8
Carbofuradan	57.30± 4.6	17.6	97.3
Inoculated control 1(C-1)	29.04± 10.3	58.3	29.04 value
Uninoculated control 2 (C-2)	69.60± 4.0	69.60 value	

Values are mean of five replicates. Mean in each column are significantly different at $p<0.05$ according to one way anova

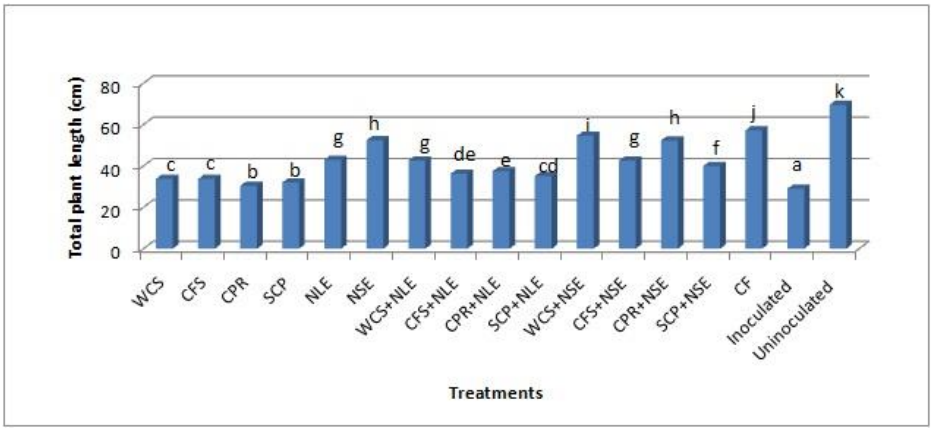

Figure 1

Showing the modulatory effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem + Bt on the total length of tomato plant infested with *M. incognita.* Values are means of five replicates. Means in each column followed by the same letter do not differ at $p<0.05$ according to Tukey's multiple range test

Table 2

Showing effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem and Bt on percentage increase/decrease in the total weight (in grams) of the tomato plant infested with *M. incognita* as compared to infected (C-1) and normal plants (C-2)

Treatments	Total plant weight (g)		
	Mean	Reduction % age	Increase %age
Neem leaf extract	8.8± 1.3	84	58.5
Neem seed extract	12.3± 2.9	77.7	120.3
Whole cell suspension	6.0± 0.9	92	8.39
Cell free supernatant	7.1± 1.5	87.1	27.5
Cell pelleted residues	6.0± 1.0	89.1	7.32
Spore/ crystal proteins	5.3± 1.0	90.4	5.35
Whole cell suspension + neem leaf extract	9.7± 1.3	82.4	74.2
Cell free supernatant + neem leaf extract	7.3± 4.6	86.7	31.9
Cell pelleted residues + neem leaf extract	7.54± 1.4	86.4	34.6
Spore/crystal proteins + neem leaf extract	7.8± 1.5	85.8	39.6
Whole cell suspension + neem seed extract	11.7± 2.0	78.8	109.8
Cell free supernatant + neem seed extract	7.3± 1.0	86.7	30.5
Cell pelleted residues + neem seed extract	8.3± 1.5	84.9	48.2
Spore/crystal proteins + neem seed extract	8.07± 3.3	85.5	44.1
Carbofuradan	19.5± 2.3	64.7	249.4
Inoculated control 1 (C-1)	5.6± 4.7	89.8	5.6 value
Uninoculated control 2 (C-2)	55.3± 3.4	55.36 value	

Values are mean of five replicates. Mean in each column are significantly different at $p<0.05$ according to one way anova

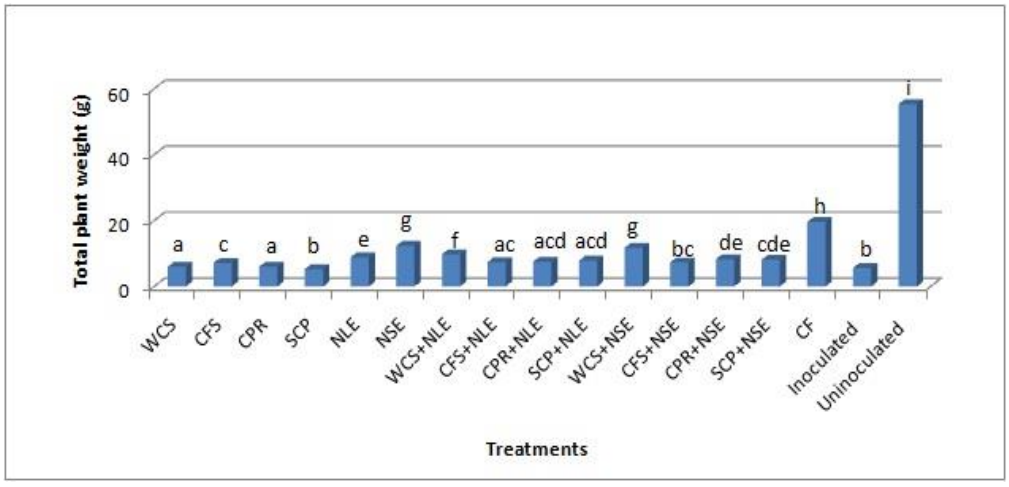

Figure 2

Showing the modulatory effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem + Bt on the total weight of tomato plant infested with *M. incognita*. Values are means of five replicates. Means in each column followed by the same letter do not differ at $p<0.05$ according to Tukey's multiple range test

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Table 3

Showing effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem and Bt on percentage reduction in the number of galls/root system of the tomato plant infested with *M. incognita* as compared to infected (C-1) and normal plants (C-2)

Treatments	Number of galls/root system	
	Mean	Reduction %age
Neem leaf extract	20.1± 1.2	7.37
Neem seed extract	20.3± 2.0	6.45
Whole cell suspension	21.70± 0.1	0.09
Cell free supernatant	20.61± 1.7	5.02
Cell pelleted residues	15.02± 1.4	30.7
Spore/ crystal proteins	20.04± 1.9	7.64
Whole cell suspension + neem leaf extract	14.96± 1.0	31.3
Cell free supernatant + neem leaf extract	18.55± 1.9	14.7
Cell pelleted residues + neem leaf extract	18.02±1.7	16.9
Spore/crystal proteins + neem leaf extract	17.2±1.8	20.7
Whole cell suspension + neem seed extract	12.56± 1.2	42.11
Cell free supernatant + neem seed extract	20.30± 1.4	6.01
Cell pelleted residues + neem seed extract	15.10± 1.1	30.41
Spore/crystal proteins + neem seed extract	20.4± 1.7	5.99
Carbofuradan 3-G	5.21± 1.3	75.5
Inoculated control 1 (C-1)	21.72± 2.4	21.72 value
Uninoculated control 2 (C-2)	0±0	

Values are mean of five replicates. Mean in each column are significantly different at $p<0.05$ according to one way anova

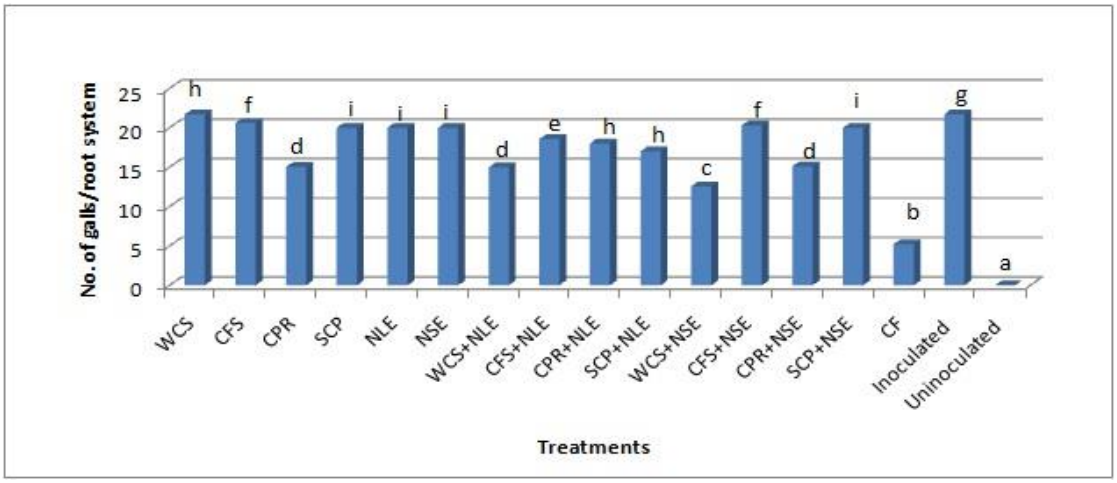

Figure 3

Showing the nematicidal effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem + Bt on the number of galls/root system of tomato plant infested with *M. incognita*. Values are means of five replicates. Means in each column followed by the same letter do not differ at $p<0.05$ according to Tukey's multiple range test

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Table 4

Showing effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem and Bt on percentage reduction in the number of egg masses/gall of the tomato plant infested with *M. incognita* as compared to infected (C-1) and normal plants (C-2)

Treatments	Number of egg masses/gall		
	Mean	Increase % age	Reduction % age
Neem leaf extract	6.63±1.0	-	34
Neem seed extract	9.46±1.4	-	6
Whole cell suspension	9.70± 1.4	-	3
Cell free supernatant	9.10± 1.1	-	9
Cell pelleted residues	5.96± 0.9	-	41
Spore/ crystal proteins	12.32± 0.8	23	-
Whole cell suspension + neem leaf extract	5.10± 0.7	-	49
Cell free supernatant + neem leaf extract	7.32± 0.9	-	27
Cell pelleted residues + neem leaf extract	5.27± 1.1	-	48
Spore/crystal proteins + neem leaf extract	11.72± 1.0	17	-
Whole cell suspension + neem seed extract	6.35± 0.8	-	37
Cell free supernatant + neem seed extract	8.31± 0.9	-	17
Cell pelleted residues + neem seed extract	6.26± 1.9	-	38
Spore/crystal proteins + neem seed extract	12.41±1.1	24	-
Carbofuradan 3-G	3.02±0.7	-	70
Inoculated control 1(C-1)	10.01±0.9	10.01 value	10.01 value
Uninoculated control 2 (C-2)	0±0		

Values are mean of five replicates. Mean in each column are significantly different at $p<0.05$ according to one way anova

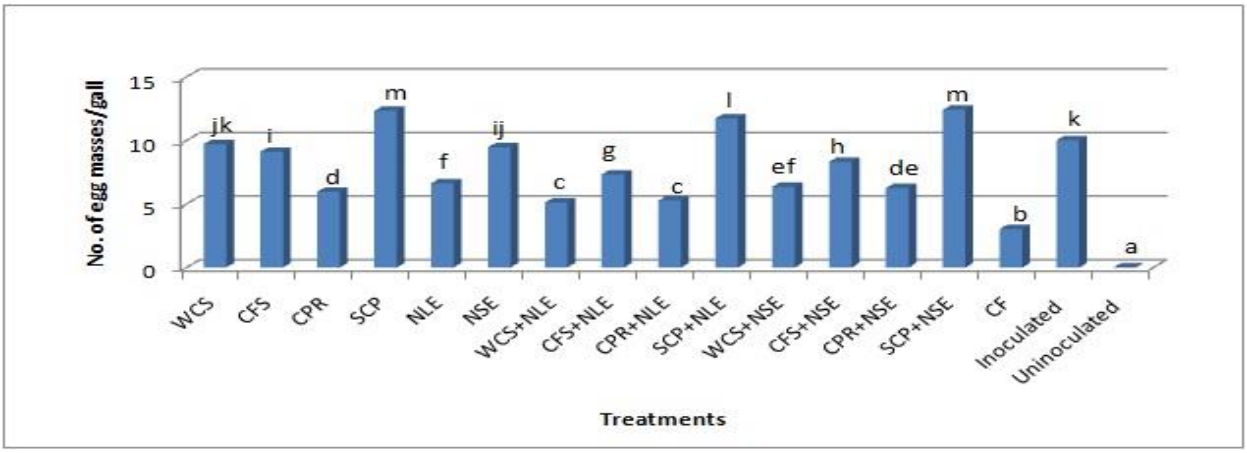

Figure 4

Showing the nematicidal effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem + Bt on the number of egg masses/gall of *M. incognita* infested tomato plants. Values are means of five replicates. Means in each column followed by the same letter do not differ at $p<0.05$ according to Tukey's multiple range test

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Table 5

Showing effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem and Bt on percentage reduction in the number of females/gall of the tomato plant infested with *M. incognita* as compared to infected (C-1) and normal plants (C-2)

	Number of females/gall		
	Mean	**Increase %age**	**Reduction %age**
Neem leaf extract	10.36± 0.9	-	31.3
Neem seed extract	11.03± 1.4	-	26.6
Whole cell suspension	9.66± 1.3	-	36
Cell free supernatant	10.39± 1.4	-	31.3
Cell pelleted residues	8.74± 0.8	-	42
Spore/ crystal proteins	15.38± 0.9	2	-
Whole cell suspension + neem leaf extract	6.63± 1.1	-	56
Cell free supernatant + neem leaf extract	9.21± 1.4	-	38.6
Cell pelleted residues + neem leaf extract	7.98± 1.1	-	48
Spore/crystal proteins + neem leaf extract	13.77± 1.1	-	8.6
Whole cell suspension + neem seed extract	7.27± 0.9	-	52
Cell free supernatant + neem seed extract	10.16± 1.4	-	32.6
Cell pelleted residues + neem seed extract	10.03± 1.3	-	33.3
Spore/crystal proteins + neem seed extract	14.70± 1.0	-	2
Carbofuradan 3-G	4.15± 0.7	-	72.3
Inoculated control 1(C-1)	15.04± 1.1	15.04 value	15.04 value
Uninoculated control 2 (C-2)	0±0		

Values are mean of five replicates. Mean in each column are significantly different at $p<0.05$ according to one way anova

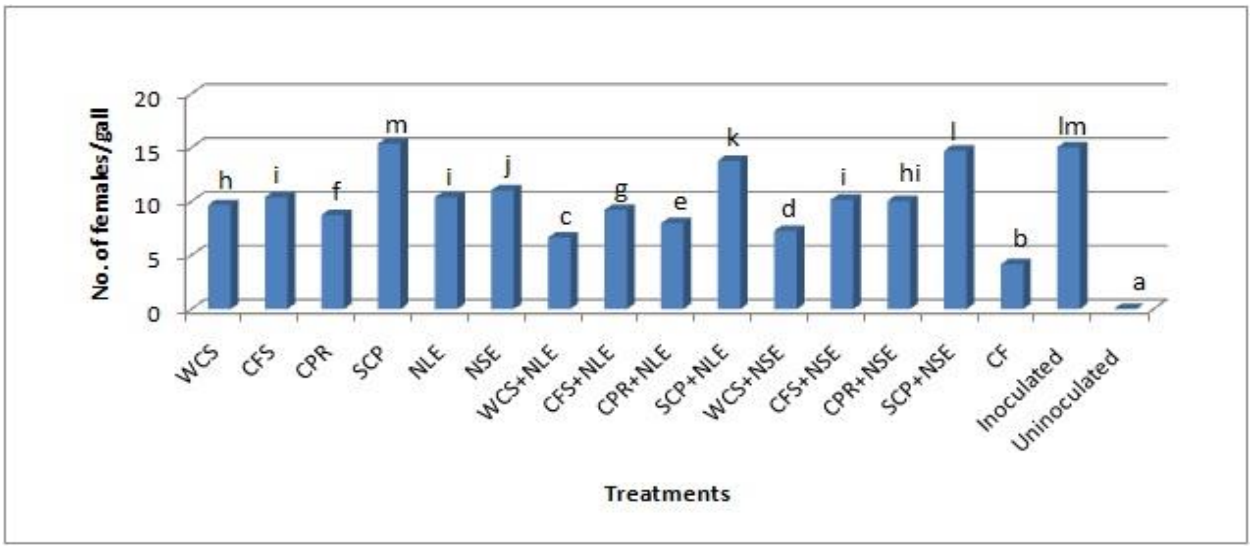

Figure 5

Showing the nematicidal effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem + Bt on the number of females/gall of *M. incognita* infested tomato plants. Values are means of five replicates. Means in each column followed by the same letter do not differ at $p<0.05$ according to Tukey's multiple range test

Table 6

Showing effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem and Bt on percentage reduction in the number of J2/1g root of the tomato plant infested with *M. incognita* as compared to infected (C-1) and normal plants (C-2)

Treatments	Number of J2/1g root	
	Mean	Reduction %age
Neem leaf extract	101.58± 3.7	65.8
Neem seed extract	151.21± 2.5	49.1
Whole cell suspension	103.63± 5.6	65.3
Cell free supernatant	113.11± 3.4	61.9
Cell pelleted residues	93.66± 3.7	68.4
Spore/ crystal proteins	150.72± 2.0	49.4
Whole cell suspension + neem leaf extract	92.22± 2.6	68.9
Cell free supernatant + neem leaf extract	101.41± 3.2	65.9
Cell pelleted residues + neem leaf extract	102.35± 4.8	65.6
Spore/crystal proteins + neem leaf extract	150.99± 2.2	49.4
Whole cell suspension + neem seed extract	97.03± 2.6	67.3
Cell free supernatant + neem seed extract	101.72± 7.9	65.7
Cell pelleted residues + neem seed extract	101.83± 3.2	65.7
Spore/crystal proteins + neem seed extract	161.58± 2.3	45.6
Carbofuradan 3-G	39.25± 4.3	86.8
Inoculated control 1 (C-1)	297.38± 39.4	297.38 value
Uninoculated control 2 (C-2)	0±0	

Values are mean of five replicates. Mean in each column are significantly different at $p<0.05$ according to one way anova

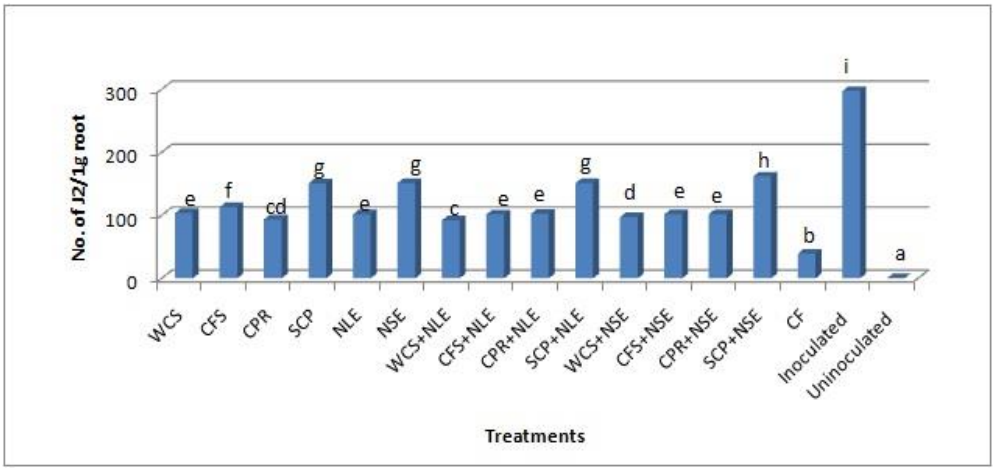

Figure 6

Showing the nematicidal effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem + Bt on the number of J2/root of *M. incognita* infested tomato plants. Values are means of five replicates. Means in each column followed by the same letter do not differ at $p<0.05$ according to Tukey's multiple range test

Table 7

Showing effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem and Bt on percentage reduction in the number of J2/250g soil of the tomato plant infested with *M. incognita* as compared to infected (C-1) and normal plants (C-2)

	Number of J2/250g soil	
	Mean	Reduction %age
Neem leaf extract	10.00± 1.1	64
Neem seed extract	15.13±8.8	45.6
Whole cell suspension	20.54± 1.8	26.2
Cell free supernatant	21.11± 1.6	24.1
Cell pelleted residues	9.00±0.8	67.6
Spore/ crystal proteins	26.38± 1.7	5.3
Whole cell suspension + neem leaf extract	10.20± 1.0	63.3
Cell free supernatant + neem leaf extract	19.69± 1.3	29.4
Cell pelleted residues + neem leaf extract	10.82± 1.3	61.1
Spore/crystal proteins + neem leaf extract	20.66± 1.7	25.8
Whole cell suspension + neem seed extract	10.36± 1.0	62.9
Cell free supernatant + neem seed extract	20.55± 1.4	26.2
Cell pelleted residues + neem seed extract	15.17± 1.0	45.6
Spore/crystal proteins + neem seed extract	25.61± 2.1	7.9
Carbofuradan 3-G	0± 0	100
Inoculated control 1 (C-1)	27.81± 2.5	27.81value
Uninoculated control 2 (C-2)	0± 0	

Values are mean of five replicates. Mean in each column are significantly different at p<0.05 according to one way anova

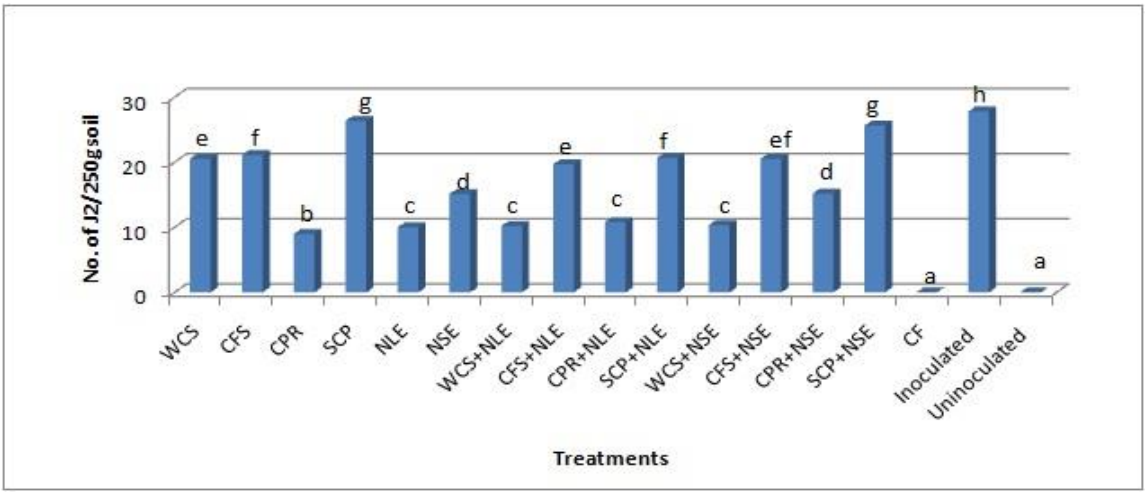

Figure 7

Showing the nematicidal effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem + Bt on the number of J2/250g soil of *M. incognita* infested tomato plants. Values are means of five replicates. Means in each column followed by the same letter do not differ at p<0.05 according to Tukey's multiple range test

Table 8

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Showing effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem and Bt on percentage reduction in the number of males/250g soil of the tomato plant infested with *M. incognita* as compared to infected (C-1) and normal

Treatments	Number of males/250g soil	
	Mean	Reduction % age
Neem leaf extract	15.41±1.1	33.3
Neem seed extract	20.73±1.7	10.3
Whole cell suspension	15.65± 1.1	32.4
Cell free supernatant	18.60± 1.4	19.4
Cell pelleted residues	15.17± 0.8	8
Spore/ crystal proteins	20.40± 1.6	11.6
Whole cell suspension + neem leaf extract	10.53± 1.4	54.5
Cell free supernatant + neem leaf extract	13.53± 1.0	41.5
Cell pelleted residues + neem leaf extract	11.42± 1.3	50.6
Spore/crystal proteins + neem leaf extract	21.07±1.9	9
Whole cell suspension + neem seed extract	8.46±1.3	63.6
Cell free supernatant + neem seed extract	14.47±1.0	37.6
Cell pelleted residues + neem seed extract	13.18±1.1	43.2
Spore/crystal proteins + neem seed extract	21.91±2.1	28.1
Carbofuradan 3-G	13.34±1.2	42.4
Inoculated control 1(C-1)	23.11± 4.0	23.11 value
Uninoculated control 2 (C-2)	0±0	

Values are mean of five replicates. Mean in each column are significantly different at $p<0.05$ according to one way anova

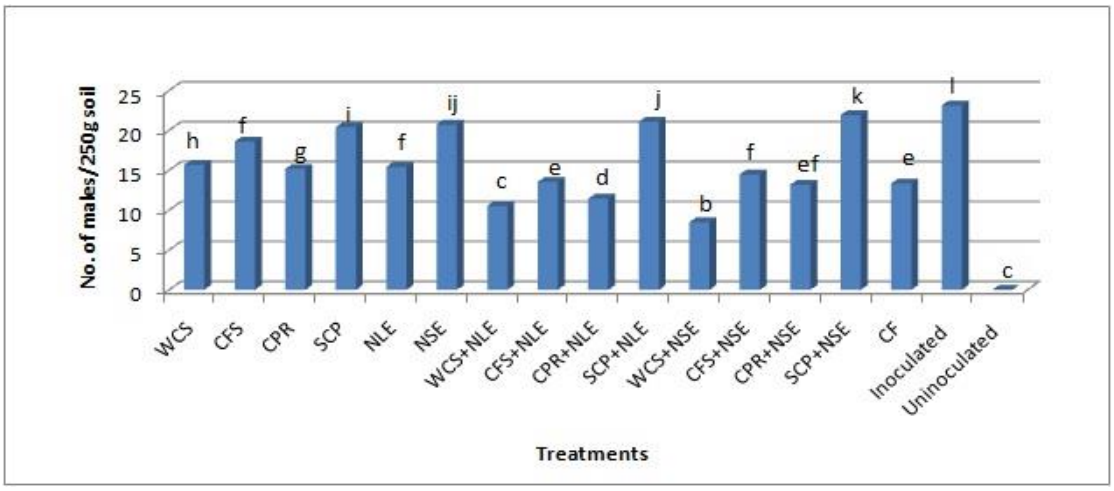

Figure 8

Showing the nematicidal effect of bare-root-dip treatment with various formulations of neem, Bt and combination of neem + Bt on the number of males/250g soil of *M. incognita* infested tomato plants. Values are means of five replicates. Means in each column followed by the same letter do not differ at $p<0.05$ according to Tukey's multiple range test

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