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Biocontrol of *Meloidogyne incognita* with Neem and Bt on Tomato Plant by Seed Dressing Treatment

Harjinder Kaur[1]✉, Harpreet Kaur[1], Praveen Rishi[2]

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.* 100% concentration of neem leaf extract, neem seed extract and 1.1×10^9 CFU/ml of Bt formulations (WCS, CFS, CPR and SCP@ 10ml/ 50 seeds) were used as seed dressing treatment to control *M. incognita* infesting tomato plants. Significant increase in shoot length, root length and total plant length was observed in NLE treated tomato plants i.e. 142.0% (52.2 cm), 211.9% (23.09 cm) and 159.0% (75.2 cm) respectively as compared to the inoculated control. Carbofuradan 3-G was also effective caused maximum increase in shoot weight, total plant weight and yield weight by 28.7% (14.8 cm), 40.6% (19.5 cm) and 286.5% (11.12 cm) respectively as compared to the inoculated control. Maximum root weight was observed in WCS+NSE i.e. 115% (5.10 cm) as compared to the inoculated control. NLE treated tomato plants showed significant increase in shoot length, root length and total plant length as compared to the uninoculated control. When CPR+NSE used as seed dressing treatment caused significant reduction 6.9% in number of galls/root system as compared to the inoculated control. Carbofuradan 3-G and WCS+NLE caused 72.3% and 54% reduction in number of females/gall respectively as compared to the inoculated control.

Key words: organic amendments, biocontrol agents, nematicidal, *M. incognita*, neem

1. INTRODUCTION

Root-knot nematodes are serious and economically most important pest of many cultivated crops around the world (Siddiqui *et al*., 2001). They are particularly damaging vegetables in tropical and subtropical countries and cause losses up to 80% in heavily infested fields (Davis *et al*., 2005).

They are among the main pathogens of tomato (*Lycopersicon esculentum* Mill) plants all over the world (Okada *et al*., 2007). Infested plants show 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 losses ranging from 28% to 68% (Browning *et al*., 2006). Therefore, there is great need to control root-knot nematodes infesting tomato plants.

Certain chemicals used as nematicides gained lot of significance when first introduced in 1940s because they served useful tools to overcome crop losses caused by nematodes. For the control of *M. incognita*, chemicals still remain to be one of the most effective methods in terms of immediate results, but phytotoxic effects sometime pose difficulties. Furthermore, many of these chemicals have proven to be carcinogens as they build up residues in plants and infiltrate into the ground water (Zukerman and Esnard, 1994). Some of these chemicals are equally hazardous to livestock, plants and also to the beneficial fauna and flora of the soil. Main limitation in the use of nematicides is that they are generally costly and not easily available besides having inherent difficulties in their handling. Therefore, there has been a growing interest in alternate disease control technologies. As modern agriculture moves to adopt more environment friendly practices there is increasing interest in the use of plants and microorganisms as source of biocontrol agents.

Cultural methods include fallowing, flooding, ploughing, use of resistant cultivars, crop rotation, organic matter amendment and green manuring. Use of organic amendment is an age-old practice and have been shown to have beneficial effects on soil nutrients, soil physical conditions, soil biological activity and crop viability (Kang *et al.,* 1981; Hungalle *et al*., 1986). A variety of plant products have been evaluated to manage plant parasitic nematodes (Chitwood, 2002). A number of bacterial genera such as *Bacillus, Pasteuria* and *Pseudomonas* (Carneira *et al*.,1998; Zareen *et al*., 2003; Vagelas *et al*., 2007; Mohammed *et al.,* 2008) and nematophagus fungi such as *Paecilomyces lilacinus, Arthrobotrys oligospora, Trichoderma harzianum, Pleurotus ostreatus, Aspergillus niger, Pythium debarianum, Fusarium oxysporum, F. moniliforme* (Mostafa, 2000; Sharon, 2001; Heydari, 2006 and Radwan, 2007; Tian *et al*., 2007) have been used as biological control agents.

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

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, Punjab 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).

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(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). The 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.

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^{0}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^{0}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. Seed dressing experiment design

Ten seeds of tomato (*Lycopersicon esculentum* cv. Pusa Ruby) were treated with standard concentration of (100%) neem leaf extract and neem seed extracts @ 10ml/50 seeds, 2.5% carbofuradan 3-G @ 10ml/50 seeds, 1.1X 10^9 CFU/ml whole cell suspension, cell free supernatant, cell pelleted residues and spore/crystal proteins @ 10ml/50 seeds and neem + Bt in combination in the ratio 1:1 using 1% gelatin as sticker. Treated seeds were sown in 15 cm diam. earthen pots each containing 6 kg soil. After germination only two seedlings were transplanted in each pot. After one week, 3600J2 of *M. incognita* were inoculated into the rhizospheric soil and pots were settled on a bench in greenhouse. Plants with nematode inoculum served as inoculated control (control-1) and without nematode inoculum as uninoculated control (control-2). Plants were uprooted at 45th day after *M. incognita* inoculation and growth parameters such as shoot weight, root weight, total plant weight, yield weight and nematode control parameters such as number of galls/root system and number females/gall were recorded (Siddiqui, 2000). Following treatment combinations were evaluated for seed dressing treatment:

i) Seed+ neem leaf extract+ pathogen
ii) Seed+ neem seed extract+ pathogen

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iii) Seed+ whole cell suspension+ pathogen
iv) Seed+ cell free supernatant+ pathogen
v) Seed+ cell pelleted residues+ pathogen
vi) Seed+ spore/crystal proteins+ pathogen
vii) Seed+ whole cell suspension+ neem leaf extract+ pathogen
viii) Seed+ whole cell suspension+ neem seed extract+ pathogen
ix) Seed+ cell free supernatant+ neem leaf extract+ pathogen
x) Seed+ cell free supernatant+ neem seed extract+ pathogen
xi) Seed+ cell pellted residues+ neem leaf extract+ pathogen
xii) Seed+ cell pelleted residues+ neem seed extract+ pathogen
xiii) Seed+ spore/crystal proteins+ neem leaf extract+ pathogen
xiv) Seed+ spore/crystal proteins+ neem seed extract+ pathogen
xv) Seed+ carbofuradan+ pathogen
xvi) Seed+ pathogen (inoculated control-1)
xvii) Seed (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 showed that seed dressing with neem leaf extract resulted in highly significant increase ($p<0.05$) in shoot length by 142.0% i.e. 52.2 cm as compared to the inoculated control (21.6 cm). CPR used as seed dressing was recorded to increase the shoot length by 58.33% (34.29 cm) as compared to the inoculated control (21.6 cm) and decrease was by 33.4% as compared to the uninoculated control (51.4 cm) (Fig. 1). Khan *et al*. (2010) reported that seed dressing treatment with cell pelleted residues of Bt-64 showed 43% increase in shoot length of okra plants infested with *M. javanica* as compared to the control and in mungbean the shoot length increased by 42%. In the present study CPR was effective and caused significant increase ($p<0.05$) in root length by 53.7% (11.3cm) followed by WCS 52.1% (11.2cm) (Table 2, Fig. 2). The modulatory effect on root length was also observed by Khan *et al*. (2010) according to which the seed dressing treatment of okra with cell pellets of Bt 64 isolate resulted in increase in root length which was more than 36% as compared to untreated control while in mungbean, maximum increase in root length was 46%.

Table 1

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

Treatments	Shoot length (cm)				
	Mean	Increase %age	Decrease %age	Increase %age	Decrease %age
Neem leaf extract	52.29± 2.6	1.5	-	142.0	-
Neem seed extract	46.46± 2.9	-	9.7	115.09	-
Whole cell suspension	34.65± 1.8	-	32.6	60.18	-
Cell free supernatant	21.14± 1.6	-	58.9	-	2.12
Cell pelleted residues	34.29±3.8	-	33.4	58.33	-
Spore/ crystal proteins	22.97± 3.6	-	55.4	6.01	-

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Whole cell suspension + neem leaf extract	38.04± 1.8	-	26	76.11	-
Cell free supernatant + neem leaf extract	21.61± 2.0	-	57.9	-	0
Cell pelleted residues + neem leaf extract	36.50± 2.3	-	28.9	68.98	-
Spore/crystal proteins + neem leaf extract	25.32± 3.3	-	50.7	17.22	-
Whole cell suspension + neem seed extract	42.18± 2.9	-	18	94.9	-
Cell free supernatant + neem seed extract	25.38± 3.2	-	50.7	17.12	-
Cell pelleted residues + neem seed extract	40.81± 2.1	-	20.6	88.9	-
Spore/crystal proteins + neem seed extract	27.07± 2.5	-	47.4	25.32	-
Carbofuradan	42.90± 4.1	-	16.5	98.61	-
Inoculated control 1(C-1)	21.66± 8.5	-	57.9	21.66 value	21.66 value
Uninoculated control 2 (C-2)	51.46± 3.4	51.46 value	51.46 value		

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

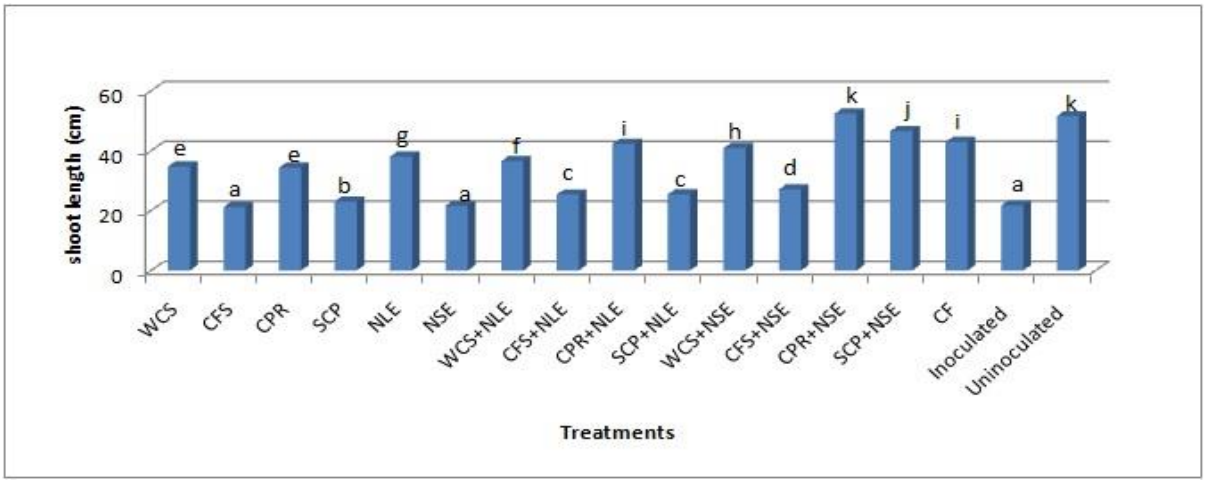

Figure 1

Showing the modulatory effect of seed dressing treatment with various formulations of neem, Bt and combination of neem + Bt on the shoot 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

NLE, NSE, WCS+NSE applied as seed dressing treatment caused 159%, 121.2% and 100.6%, highly significant increase (p<0.05) in total plant length i.e. 75.2cm, 64.2cm and 58.2cm respectively as compared to the inoculated control (29.04cm) (Table 3, Fig. 3). Serfoji *et al.* (2010) also observed that seed dressing of tomato with combination of mycorrhizal fungus- *Glomus aggregatum*, vermicompost and whole cell suspension of *Bacillus coagulans* caused maximum increase in total plant length by 36.02 cm. Seed dressing with carbofuradan 3-G caused 28.7% increase in shoot weight i.e. 14.8g as compared to the inoculated control (11.5g) (Table 4, Fig. 4). In conformity with our results, Fatema and Ahmad (2005) observed that furadan 3-G caused maximum increase in

shoot weight (26.2g) followed by neem oil (18.03g) as compared to the control (5.08g) when used as seed dressing treatment. Mukhopadhyay and Roy (2007) indicated that in seed dressing treatment of cowpea with T7 (carbosulfan 25DS at 3.0% w/w + neem cake, @1000kg/ha) caused greatest fresh shoot weight over the untreated check i.e. 194.1g, followed by T8 (carbosulfan 25EC at 0.1% for 4 hr+neem cake, @1000kg/ha) i.e 179.8g.

Table 2

Showing effect of seed dressing treatment with various formulations of neem, Bt and combination of neem + Bt on percentage increase/decrease in the root length (in cm) the tomato plant as compared to infected (C-1) and normal plants (C-2)

Treatments	Root length (cm)				
	Mean	Increase %age	Decrease %age	Increase %age	Decrease %age
Neem leaf extract	23.02±3.4	27	-	211.9	-
Neem seed extract	17.79±1.2	-	2.2	139.8	-
Whole cell suspension	11.23±1.2	-	38.1	52.16	-
Cell free supernatant	7.05±1.0	-	61	-	4.47
Cell pelleted residues	11.35±1.4	-	37.5	53.7	-
Spore/ crystal proteins	7.26±0.9	-	59.8	-	1.62
Whole cell suspension + neem leaf extract	15.22±1.1	-	16.02	106.23	-
Cell free supernatant + neem leaf extract	9.93±1.2	-	45.3	34.55	-
Cell pelleted residues + neem leaf extract	12.65±1.3	-	30.3	71.40	-
Spore/crystal proteins + neem leaf extract	8.11±1.2	-	55.2	9.89	-
Whole cell suspension + neem seed extract	16.08±1.4	-	11.6	117.8	-
Cell free supernatant + neem seed extract	10.55±1.2	-	41.9	42.95	-
Cell pelleted residues + neem seed extract	13.15±2.1	-	27.6	78.18	-
Spore/crystal proteins + neem seed extract	7.85±0.9	-	56.9	6.36	-
Carbofuradan	14.40± 1.1	-	20.4	95.12	-
Inoculated control 1(C-1)	7.38± 2.4	-	59.6	7.38 value	7.38 value
Uninoculated control 2 (C-2)	18.15± 1.5	18.15 value	18.15value		

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

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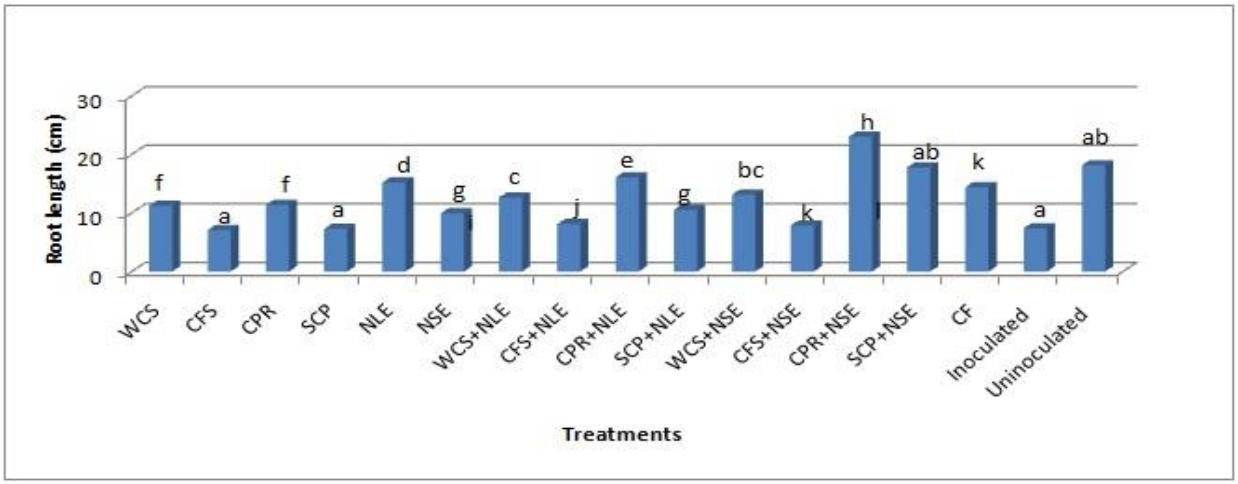

Figure 2

Showing the modulatory effect of seed dressing treatment with various formulations of neem, Bt and combination of neem + Bt on the root 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 3

Showing effect of seed dressing treatment with various formulations of neem, Bt and combination of neem + 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	Increase %age	Decrease %age	Increase %age	Decrease %age
Neem leaf extract	75.22± 0.3	8.04	-	159.02	-
Neem seed extract	64.26± 0.2	-	7.7	121.2	-
Whole cell suspension	45.88± 2.3	-	34.1	57.98	-
Cell free supernatant	28.2± 2.0	-	59.4	-	2.89
Cell pelleted residues	45.66± 2.5	-	34.4	57.23	-
Spore/ crystal proteins	30.23± 3.8	-	56.6	4.09	-
Whole cell suspension + neem leaf extract	55.7± 2.0	-	19.9	-	31.47
Cell free supernatant + neem leaf extract	31.43± 2.8	-	54.8	8.23	-
Cell pelleted residues + neem leaf extract	49.16± 2.6	-	29.4	60.2	-
Spore/crystal proteins + neem leaf extract	33.56± 3.9	-	51.8	15.56	-
Whole cell suspension + neem seed extract	58.27± 3.2	-	16.3	100.65	-

Cell free supernatant + neem seed extract	36.03± 3.7	-	48.2	24.07	-
Cell pelleted residues + neem seed extract	53.84± 3.3	-	22.7	-	21.83
Spore/crystal proteins + neem seed extract	34.92± 2.6	-	49.8	20.2	-
Carbofuradan	57.30± 0.3	-	17.6	97.31	-
Inoculated control 1(C-1)	29.04± 0.7	-	58.3	29.04 Value	29.04 Value
Uninoculated control 2 (C-2)	69.60± 0.2	69.60 Value	69.60 value		

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

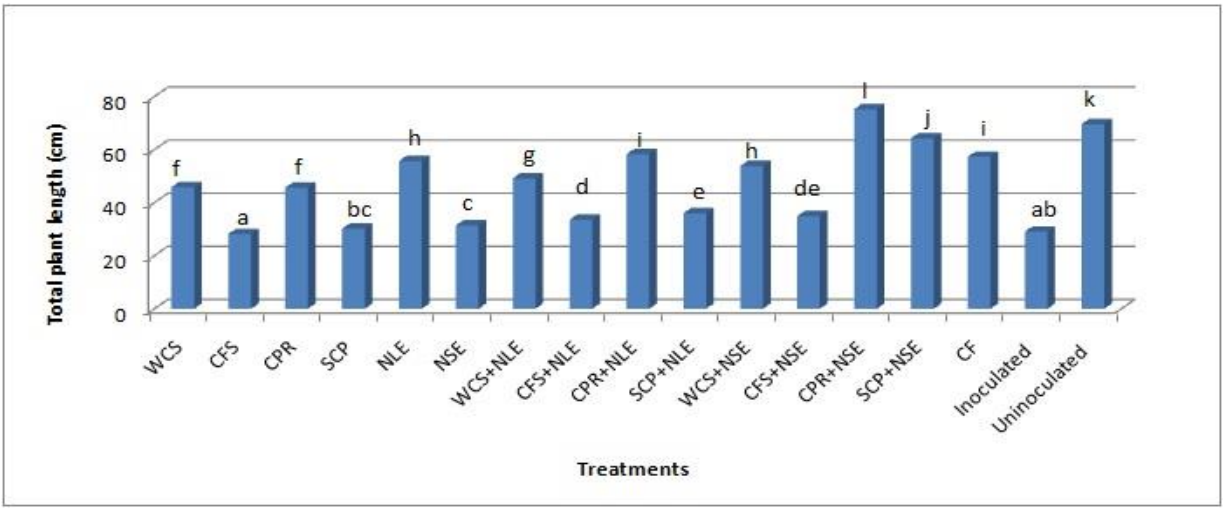

Figure 3

Showing the modulatory effect of seed dressing 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 4

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

Treatments	Shoot weight (g)			
	Mean	Decrease %age	Increase %age	Decrease %age
Neem leaf extract	8.80±1.2	81.5	-	23.74
Neem seed extract	9.51±0.9	80.1	-	17.5
Whole cell suspension	6.11±0.9	87.2	-	47.05

Cell free supernatant	4.60±1.1	90.3	-	60.1
Cell pelleted residues	4.64±1.2	90.3	-	59.79
Spore/ crystal proteins	3.16±0.7	93.5	-	72.61
Whole cell suspension + neem leaf extract	6.14±1.0	87.2	-	46.79
Cell free supernatant + neem leaf extract	4.40±1.1	90.7	-	61.87
Cell pelleted residues + neem leaf extract	4.43±0.9	90.7	-	61.61
Spore/crystal proteins + neem leaf extract	4.23±0.9	91.2	-	63.34
Whole cell suspension + neem seed extract	7.12±0.9	85.1	-	38.30
Cell free supernatant + neem seed extract	5.84±1.2	87.8	-	49.39
Cell pelleted residues + neem seed extract	5.80±0.9	87.8	-	49.39
Spore/crystal proteins + neem seed extract	4.67±1.2	90.3	-	59.53
Carbofuradan	14.86±1.4	69	28.7	-
Inoculated control 1(C-1)	11.54±4.1	75.9	11.54 value	11.54 Value
Uninoculated control 2 (C-2)	47.82±2.7	47.82 value		

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

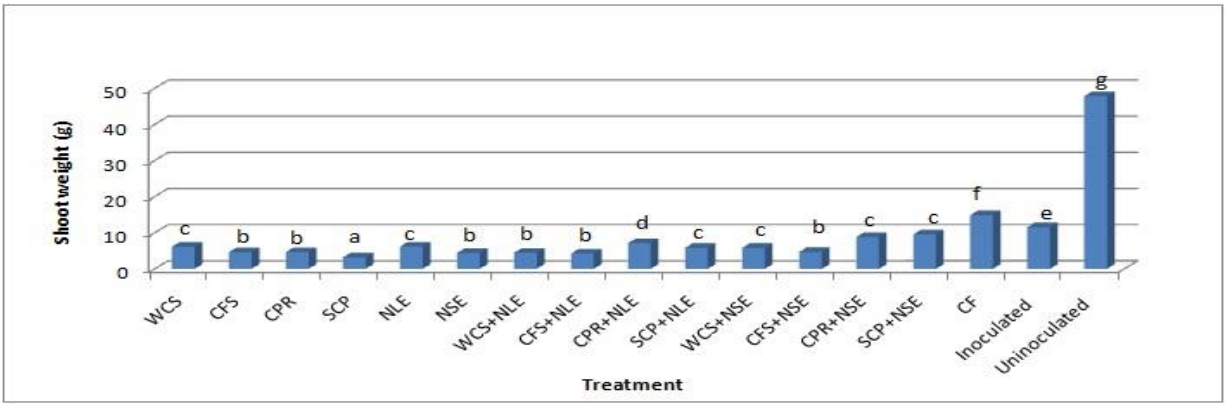

Figure 4

Showing the modulatory effect of seed dressing treatment with various formulations of neem, Bt and combination of neem + Bt on the shoot 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 5

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

Treatments	Root weight (g)			
	Mean	Decrease %age	Increase %age	Decrease %age
Neem leaf extract	3.08±0.7	60	29.95	-
Neem seed extract	3.58±0.9	53.3	51.05	-
Whole cell suspension	3.32±0.6	56	40.08	-
Cell free supernatant	2.59±0.7	66.6	9.28	-
Cell pelleted residues	3.49±1.0	54.6	47.25	-
Spore/ crystal proteins	1.86±0.6	76	-	21.51
Whole cell suspension + neem leaf extract	4.67±0.9	38.6	97.04	-
Cell free supernatant + neem leaf extract	3.76±1.1	50.6	58.64	-
Cell pelleted residues + neem leaf extract	3.97±0.9	48	67.5	-
Spore/crystal proteins + neem leaf extract	3.32±0.9	56	40.08	-
Whole cell suspension + neem seed extract	5.10±1.4	32	115.1	-
Cell free supernatant + neem seed extract	3.54±0.7	53.3	49.36	-
Cell pelleted residues + neem seed extract	3.39±0.7	48	43.03	-
Spore/crystal proteins + neem seed extract	3.25±0.8	57.33	37.13	-
Carbofuradan	4.61±0.8	38.6	94.5	-
Inoculated control 1 (C-1)	2.37±0.8	69.3	2.37 value	2.37 value
Uninoculated control 2 (C-2)	7.58±0.7	7.58 value		

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

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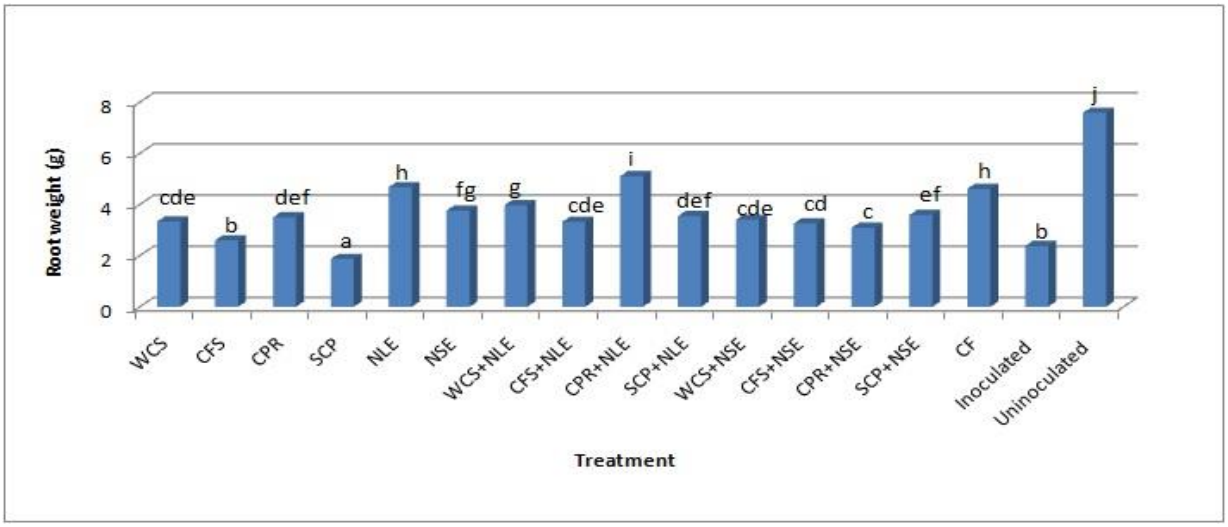

Figure 5

Showing the modulatory effect of seed dressing treatment with various formulations of neem, Bt and combination of neem + Bt on the root 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

Table 6

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

Treatments	Total plant weight (g)			
	Mean	Decrease %age	Decrease %age	Increase %age
Neem leaf extract	8.80±1.4	84	36.7	-
Neem seed extract	9.51±1.4	82.8	31.7	-
Whole cell suspension	9.44±1.1	82.9	32.1	-
Cell free supernatant	7.20±1.4	86.9	48.2	-
Cell pelleted residues	8.13±1.6	85.3	41.5	-
Spore/ crystal proteins	5.02±0.9	90.9	63.9	-
Whole cell suspension + neem leaf extract	11.25±5.9	79.7	19.4	-
Cell free supernatant + neem leaf extract	8.16±1.5	85.3	41.3	-
Cell pelleted residues + neem leaf extract	8.40±1.3	84.8	39.6	-
Spore/crystal proteins + neem leaf extract	7.56±1.3	86.4	45.6	-

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Whole cell suspension + neem seed extract	12.22±1.7	77.9	12.2	-
Cell free supernatant + neem seed extract	9.80±6.0	82.2	29.5	-
Cell pelleted residues + neem seed extract	9.18±1.2	83.5	34	-
Spore/crystal proteins + neem seed extract	7.99±1.8	85.7	42.5	-
Carbofuradan	19.57±2.3	64.7	-	40.6
Inoculated control 1 (C-1)	13.91±4.7	74.8	13.91 value	13.91 value
Uninoculated control 2 (C-2)	55.36±3.3	55.36 value		

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

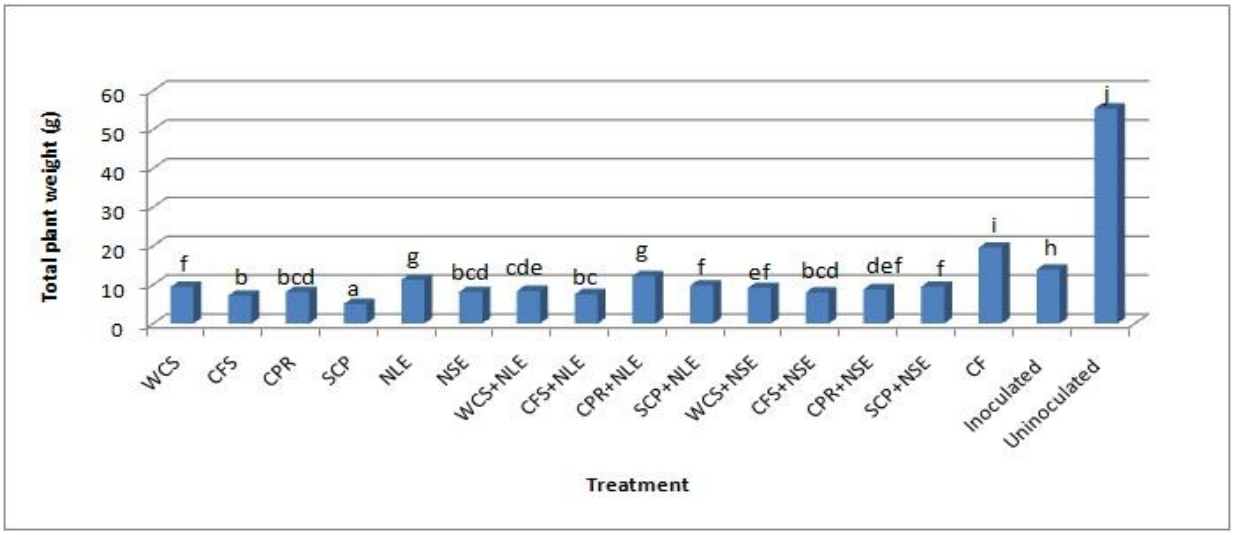

Figure 6

Showing the modulatory effect of seed dressing 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

The seed treatment with SCP with also resulted in significant increase ($p<0.05$) in shoot weight by 72.6% (3.1g) as compared to the inoculated control (11.5g). Khan *et al*. (2010) also reported that in seed dressing treatment with cell pelleted residues of Bt-64 isolate the shoot weight of okra was significantly increased ($p<0.05$) by 35% while in mungbean maximum increase was by 97%. Seed dressing with WCS+NSE caused increase in root weight by 115.1% (5.1g) as compared to the inoculated control (2.3g) (Table 5, Fig. 5). Furthermore, out of Bt alone formulations, CPR was less effective and caused significant increase in root weight by 47.2% (3.49g) as compared to the inoculated control. However, Khan *et al*. (2010) observed that seed treatment of okra with cell pelleted residues of Bt-64 strain increased root weight by 72% as compared to the untreated control while in mungbean, root weight data revealed 71% increase as compared to the control. Seed dressing with carbofuradan 3-G caused 40.6% (19.5g) increase in total plant weight as compared to the inoculated control (13.9g) (Table 6, Fig. 6). Seed dressing with all the formulations of neem, Bt, neem + Bt were ineffective and caused significant decrease ($p<0.05$) in total plant weight than the inoculated control.

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Table 7

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

Treatments	Yield weight (g)			
	Mean	Decrease %age	Increase %age	Decrease %age
Neem leaf extract	3.54± 0.9	72.8	22.9	-
Neem seed extract	3.53± 5.0	72.8	22.5	-
Whole cell suspension	3.67± 0.9	72	27.4	-
Cell free supernatant	2.61± 0.6	79.8	-	9.37
Cell pelleted residues	4.31± 1.0	66.6	49.6	-
Spore/ crystal proteins	3.23± 0.8	75.1	12.1	-
Whole cell suspension + neem leaf extract	5.71± 0.9	55.8	98.2	-
Cell free supernatant + neem leaf extract	3.54± 0.8	72.8	22.9	-
Cell pelleted residues + neem leaf extract	3.23± 0.8	75.1	12.1	-
Spore/crystal proteins + neem leaf extract	1.75± 0.4	86.8	-	39.23
Whole cell suspension + neem seed extract	5.27± 0.9	59.6	82.9	-
Cell free supernatant + neem seed extract	3.18± 0.7	75.9	10.4	-
Cell pelleted residues + neem seed extract	2.99± 0.8	77.5	3.8	-
Spore/crystal proteins + neem seed extract	2.18± 0.8	83.7	-	24.3
Carbofuradan	11.12± 1.8	13.9	286.1	-
Inoculated control 1(C-1)	2.88± 8.3	78.2	2.88 Value	2.88 Value
Uninoculated control 2 (C-2)	12.94± 2.5	12.94 value		

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

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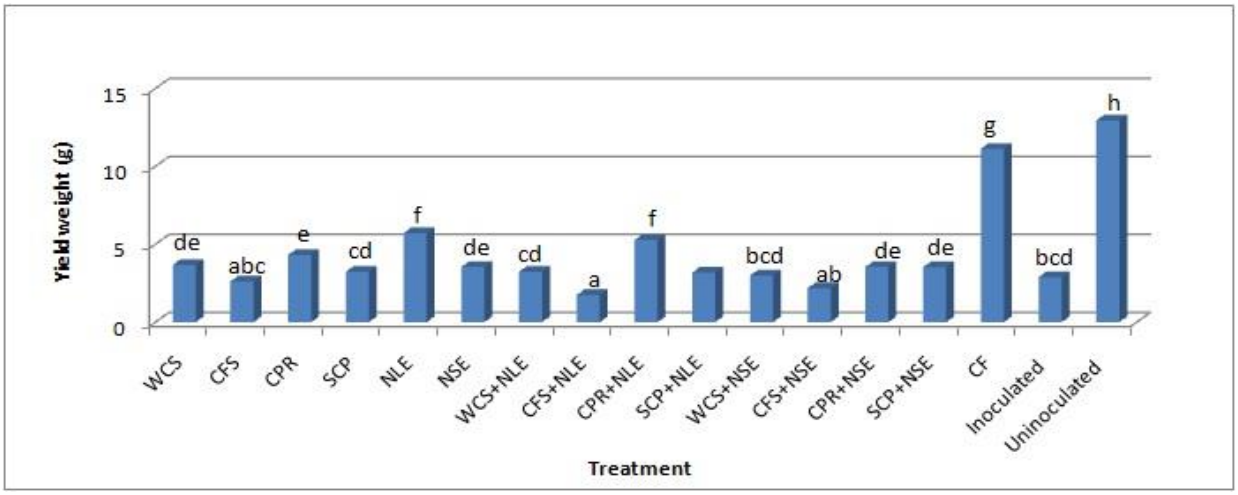

Figure 7

Showing the modulatory effect of seed dressing treatment with various formulations of neem, Bt and combination of neem + Bt on the yield 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

Table 8

Showing effect of seed dressing treatment with various formulations of neem, Bt and combination of neem + 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	Increase %age	Reduction %age
Neem leaf extract	21.62± 2.5	-	0.46
Neem seed extract	25.94± 2.0	19.3	-
Whole cell suspension	45.88± 2.8	111	-
Cell free supernatant	24.50± 2.6	12.9	-
Cell pelleted residues	24.16± 4.0	11	-
Spore/ crystal proteins	25.90± 2.7	19.3	-
Whole cell suspension + neem leaf extract	21.41± 1.9	-	1.3
Cell free supernatant + neem leaf extract	21.35± 2.1	-	1.8
Cell pelleted residues + neem leaf extract	19.63± 2.5	-	9.6
Spore/crystal proteins + neem leaf extract	24.03± 3.8	10.5	-
Whole cell suspension + neem seed extract	27.06± 3.0	24.4	-

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Cell free supernatant + neem seed extract	20.98± 2.2	-	3.6
Cell pelleted residues + neem seed extract	20.29± 1.8	-	6.9
Spore/crystal proteins + neem seed extract	23.45± 4.1	7.8	-
Carbofuradan 3-G	5.21± 1.3	-	75.9
Inoculated control 1(C-1)	21.72± 2.4	21.72283 value	21.72283 value
Uninoculated control 2 (C-2)	0±0	0	

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

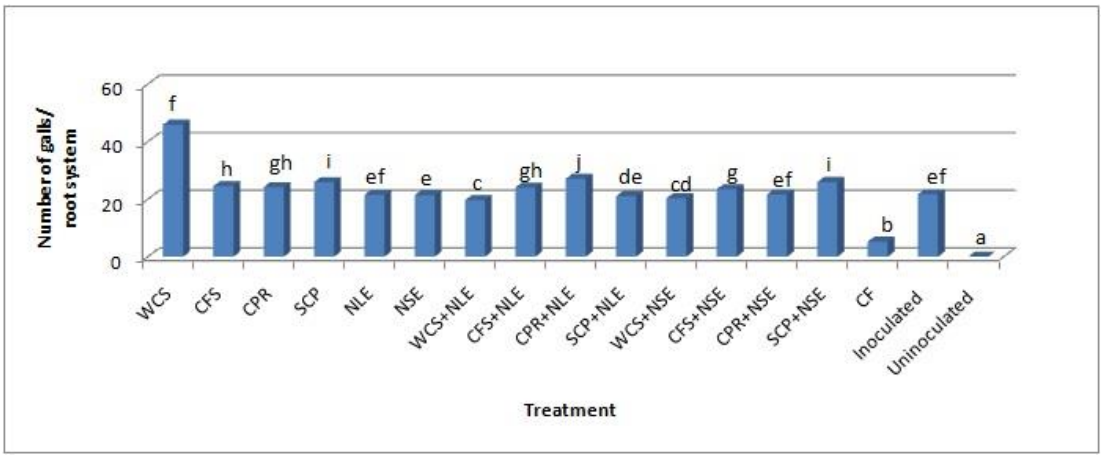

Figure 8

Showing the nematicidal effect of seed dressing 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

Table 9

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

Treatments	Number of females/gall	
	Mean	Reduction %age
Neem leaf extract	11.01± 1.9	26.6
Neem seed extract	10.50± 1.1	30
Whole cell suspension	10.61± 1.7	29.3
Cell free supernatant	7.75± 1.3	48.6

Cell pelleted residues	9.20± 1.5	38.6
Spore/ crystal proteins	10.53± 1.1	30
Whole cell suspension + neem leaf extract	6.95± 1.1	54
Cell free supernatant + neem leaf extract	9.32± 1.3	38
Cell pelleted residues + neem leaf extract	9.39± 1.5	38
Spore/crystal proteins + neem leaf extract	10.79± 1.6	28.6
Whole cell suspension + neem seed extract	8.53± 1.1	43.3
Cell free supernatant + neem seed extract	10.40± 1.4	30.6
Cell pelleted residues + neem seed extract	9.47± 1.1	37.3
Spore/crystal proteins + neem seed extract	10.52± 1.7	30
Carbofuradan 3-G	4.15± 0.7	72.3
Inoculated control 1 (C-1)	15.048± 1.1	15.04891 value
Uninoculated control 2 (C-2)	0±0	

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

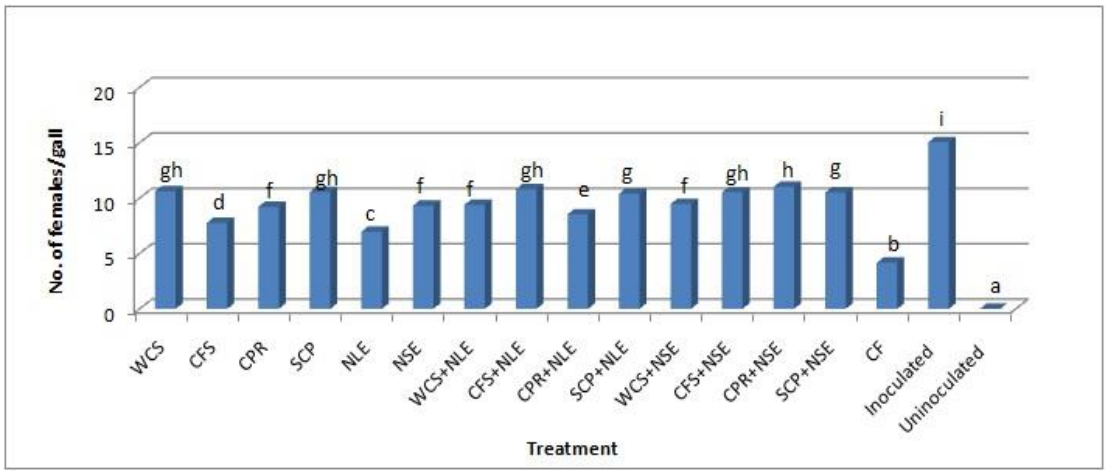

Figure 9

Showing the nematicidal effect of seed dressing 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

Serfoji *et al.* (2010) observed that seed dressing treatment of tomato with soil inhabiting fungus, *Glomus aggregatum*+ vermicompost caused 80.5% increase in total plant weight as compared to the infested soil control (51.3g). Seed dressing with carbofuradan 3-G and WCS+NLE caused 286.1% (11.2g) and 98.2% (5.71g) followed by Whole cell suspension + neem seed extract caused 82.9% (5.27g) increase in yield weight as compared to the inoculated control (Table 7, Fig. 7).

The present study indicated that CPR+NSE used as seed dressing treatment caused significant reduction 6.9% in number of galls/root system as compared to the inoculated control (75.9%) (Table 8, Fig. 8). In contrast, Khan et al. (2010) seed dressing treatment with cell pelleted residues formulation of different strains of Bt. Bt-64 isolate caused maximum reduction in gall formation by 76%, followed by Bt-14 showing a reduction in number of galls/root system by greater than 59% as compared to the control. Seed dressing with carbofuradan 3-G and WCS+NLE caused 72.3% and 54% reduction in number of females/gall respectively as compared to the inoculated control (Table 9, Fig. 9).

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Declaration of conflicting interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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