ISSN 2347 – 3819 EISSN 2347 – 386X

discovery Agriculture

Nematicidal effect of neem and Bt on *Meloidogyne incognita* infesting tomato plant by seed dressing treatment

Kaur H[1]*, Kaur H[2], Rishi P[3]

1. Guru Nanak College, Department of Zoology, Budhlada, Mansa-151502, Punjab, India
2. Department of Zoology and Environmental Sciences, Punjabi University, Patiala, Punjab – 147 002, India
3. Department of Microbiology, Punjab University, Chandigarh, Punjab-India

***Corresponding author:** Guru Nanak College, Department of Zoology, Budhlada, Mansa-151502, Punjab; E-mail: kamalsranharjinder@gmail.com

Publication History
Received: 23 June 2013
Accepted: 14 July 2014
Published: 1 August 2014

Citation
Kaur H, Kaur H, Rishi P. Nematicidal effect of neem and Bt on *Meloidogyne incognita* infesting tomato plant by seed dressing treatment. *Discovery Agriculture*, 2014, 2(6), 28-40

ABSTRACT

Seed dressing treatment with CPR+NLE was effective and caused significant reduction in number of galls/root system and number of males/250g soil i.e. 9.6% as compared to the inoculated control. WCS+NLE was effective to reduce the number of J2/1g root, number of J2/250g soil, number of egg masses/gall and number of females/gall i.e. 39%, 27.6%, 32% and 54% respectively as compared to the inoculated control. WCS+NLE and WCS+NSE were equally effective to reduce the number of egg masses/gall. Seed dressing with Carbofuradan 3 G was most effective to reduce the number of gall/root system, number of J2/1g root, number of J2/250g soil, number of males/250g soil, number of egg masses/gall and number of females/gall i.e. 9.6%, 86.9%, 100%, 42.2%, 70% and 72.3% respectively as compared to the inoculated control.

Key words: *Meloidogyne incognita*, *Bacillus thuringiensis,* neem, eggs, carbofuradan 3 G

Abbreviations: CPR+NLE- cell pelleted residues+neem leaf extract; WCS+NLE- whole cell suspension+neem leaf extract; WCS+NSE- whole cell suspension+neem seed extract

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).

Root-knot nematodes are serious and economically most important pest of many cultivated crops specially tomato around the world. 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.

Several methods are known to manage the root-knot nematode. They include the use of nematicides, organic amendments, resistant cultivars, soil solarization and biological control, which have been used with different levels of success on tomato (Randhawa et al., 2001; Sakhuja and Jain, 2001). 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

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 et al., 2008).

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 et al., 2008).

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 et al., 2008).

Kaur et al.
Nematicidal effect of neem and Bt on *Meloidogyne incognita* infesting tomato plant by seed dressing treatment,
Discovery Agriculture, 2014, 2(6), 28-40,

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^{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. 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 nematode control parameters such as number of galls/root system, number of J2/1g root, number of J2/250g soil, number of males/250g soil, number of egg masses/gall and number of females/gall and growth parameters such as total plant length, total plant weight 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
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

Kaur et al.
Nematicidal effect of neem and Bt on *Meloidogyne incognita* infesting tomato plant by seed dressing treatment,
Discovery Agriculture, 2014, 2(6), 28-40,

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

The efficacy of CPR+NLE as seed dressing treatment in reducing number of galls/root system i.e. 9.6% as compared to the inoculated control (Table 1, Figure 1). 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. While in mungbean, significant reduction in number of galls/root system was 79% in Bt-64 isolate. Seed dressing treatment with NLE was ineffective and caused reduction in number of galls/root system by only 0.46% as compared to inoculated control. Carbofuradan 3-G used as seed dressing treatment caused significant reduction by 75.9% in number of galls/root system as compared to the inoculated control (Table 1, Figure 1). Similar results have been reported by Fatema and Ahmed (2005) that furadan 3G was most effective to reduce the galling incidence as well as nematode development within the treated plants. However, neem oil gave better results in reduction of nodulation with lower galling incidence followed by garlic bulb extract. The maximum reduction in number of J2/1g root was caused by carbofuradan 3 G i.e. 86.9% as compared to the inoculated control, followed by WCS+NLE and WCS i.e. 39.3% and 31.9% respectively (Table 2, Figure 2). In support of the present findings, Fatema and Ahmad (2005) had also demonstrated that furadan 3G gave best results to decrease the number of J2/10 galls followed by neem oil and garlic extract. Earlier workers such as Oostendorp and Sikoora (1990) and Racke and Sikora (1992) reported that rhizospheric use of bacteria as seed treatment reduced nematode penetration to root system.

Seed dressing with carbofuradan 3-G, WCS+NLE, CFS+NLE, CPR+NLE and WCS caused 100%, 27.6%, 26.9%, 26.6% and 25.8% reduction in number of J2/250g soil as compared to the inoculated control (Table 3, Figure 3). Khan et al. (2010) reported that seed dressing treatment of okra with cell pelleted residues of Bt-64 caused more reduction in nematode population/200g soil as compared to the untreated control and in mungbean maximum reduction by 51% was recorded. Serfoji et al. (2010) reported that seed dressing treatment of tomato with vermicompost caused reduction of nematode population i.e. 486.4% /250 cc soil as compared to the infested soil control. Seed dressing with carbofuradan 3-G, CPR+NSE and CPR+NLE caused 42.2%, 9.5% and 8.6% reduction in number of males/250g soil respectively as compared to inoculated control (Table 4, Figure 4). CPR+NSE used as seed dressing treatment was effective and resulted in 9.5% decrease in number of males/250g soil as compared to the inoculated control.

Carbofuradan 3-G caused 70% reduction in number of egg masses/gall as compared to the inoculated control (Table 5, Figure 5). Fatema and Ahmed (2005) reported that maximum reduction in egg masses/10 galls was observed on plants treated with furadan 3G followed by neem oil and garlic extracts. In the present study, CFS used as seed dressing treatment caused significant reduction by 32% in number of egg masses/gall as compared to the inoculated control. Khan et al. (2010) reported that seed dressing treatment of okra with cell pelleted residues of different strains of Bt were nematicidal. Bt-64 isolate was recorded to be most effective and caused maximum reduction in egg masses/root system by 64%. While in mungbean reduction was by 59% as compared to the untreated control. Serfoji et al. (2010) observed that seed dressing treatment with vermicompost + whole cell suspension of *B. coagulans* caused maximum reduction in egg masses/plant i.e. 32.08% as compared to the infested soil control. Mukhopadhyay and Roy (2007) recorded that seed dressing of cowpea with T7 (carbosulfan 25 DS at 3% + neem cake @ 1000 kg/ha) at planting caused maximum reduction in final egg mass index as compared to the untreated 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 6, Figure 6). 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 7, Figure 7). 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 40.6% (19.5g) increase in total plant weight as compared to

Kaur et al.
Nematicidal effect of neem and Bt on *Meloidogyne incognita* infesting tomato plant by seed dressing treatment,
Discovery Agriculture, 2014, 2(6), 28-40,

the inoculated control (13.9g) (Table 8, Figure 8). 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.

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).

REFERENCES

1. Adesiyan SO, Caveness FE, Adeniji MO, Fawole B. Nematode pest of tropical crops. Heinemann Educational Books (Nig.) Plc. 1990
2. Akhtar M, Mahmood I. Nematode populations and short-term tomato growth in response to neem-based products and others soil amendments. *Nematropica,* 1994, 24, 169-173
3. Fatema S, Ahmed MU. Comparative efficacy of some organic amendments and a nematicide (Furadan-3G) against root-knot on two local varieties of groundnut. *Plant Pathol,* 2005, 4 (1), 54-57
4. Juss A. Plant name details. *Mem Mus Hist Nat*, 1830, 19, 221
5. Khan MQ, Abbasi MW, Zaki MJ, Khan SA. Evaluation of *Bacillus thuringiensis* isolates against root-knot nematodes following seed application in okra and mungbean. *Pakistan J Botany*, 2010, 42 (4), 2903-2910
6. Mastol RE, Chhonkara PK, Singh AD, Patra AK. Changes in soil biological and biochemical characteristics in a long-term field trial on a subtropical inceptisol. *Soil biology and Biochemistry*, (2006), 38 : 1577-1582
7. Mohammed SH, Saedy MAE, Enan MR, Ibrahim NE, Ghereeb A, Salah AM. Biocontrol efficiency of *Bacillus thuringiensis* toxins against root knot nematode, *Meloidogyne incognita*. *J Cell Mol Biol,* 2008, 7 (1), 57-66
8. Mukhopadhyay IAK, Roy K. Management of reniform nematode on cowpea,*Vigna unguiculata*. *Nematol Mediterr*, 2007, 35, 183-191
9. Racke J, Sikora RA. Isolation, formulation and antagonistic activity of rhizobacteria toward the potato cyst nematode *Globodera pallida*. *Soil Biol Biochem*, 1992, 24, 521-526
10. Randhawa N, Sakhuja PK, Singh I. Management of root-knot nematode *Meloidogyne incognita* in tomato with organic amendments. *Plant Dis Res,* 2001, 16, 274-276
11. Rao MS, Parvatha RP, Mittal A, Chandravadana MV, Nagesh M. Effect of some secondary plant metabolites on seed treatment agents against *Meloidogyne incognita* on tomato. *Nematol Mediterr,* 1996, 24, 49-51
12. Sakhuja PK, Jain RK. Nematode diseases of vegetable crops and their management. In: *Diseases of fruits and vegetables and their management*. (Thind, T. S. ed.), Kalyani Pub., Ludhiana, India, 2001, 474
13. Sasser JN, Carter CC, Hartman KM. Standardization of host suitability studies and reporting of resistance to root-knot nematode. *Crop nematode Research and Control Project Raleigh*, North Carolina, 1984, 7
14. Serfoji, P., Rajeshkumar, S. & Selvaraj, T. Management of root-knot nematode, *Meloidogyne incognita* on tomato cv Pusa Ruby by using vermicompost, AM fungus, *Glomus aggregatum* and mycorrhiza helper bacterium, *Bacillus coagulans*. *J Agric Technol,* 2010, 6 (1), 37-45
15. Siddiqui, M. R. *Tylenchida: Parasites of plants and Insects*, 2nd edn. CABI Publishing UK, Wallingford (GB). 2000, 833
16. Singh P, Siddiqui ZA. Biocontrol of root knot nematode *Meloidogyne incognita* by the isolates of *Bacillus* on tomato. *Archives Phytopathology and Plant Protection,* (2010), 43 (6): 552-561
17. Sivakumar M, Gunasekaran K. Management of root-knot nematodes in tomato, chilli and brinjal by neem oil formulations. *J Biopest*, 2011, 4 (2), 198-200
18. Williamson VM, Hussy RS. Nematode pathogenesis and resistance in plants. *Plant Cell*, 1996, 8: 1735-1745

Table 1

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	-
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

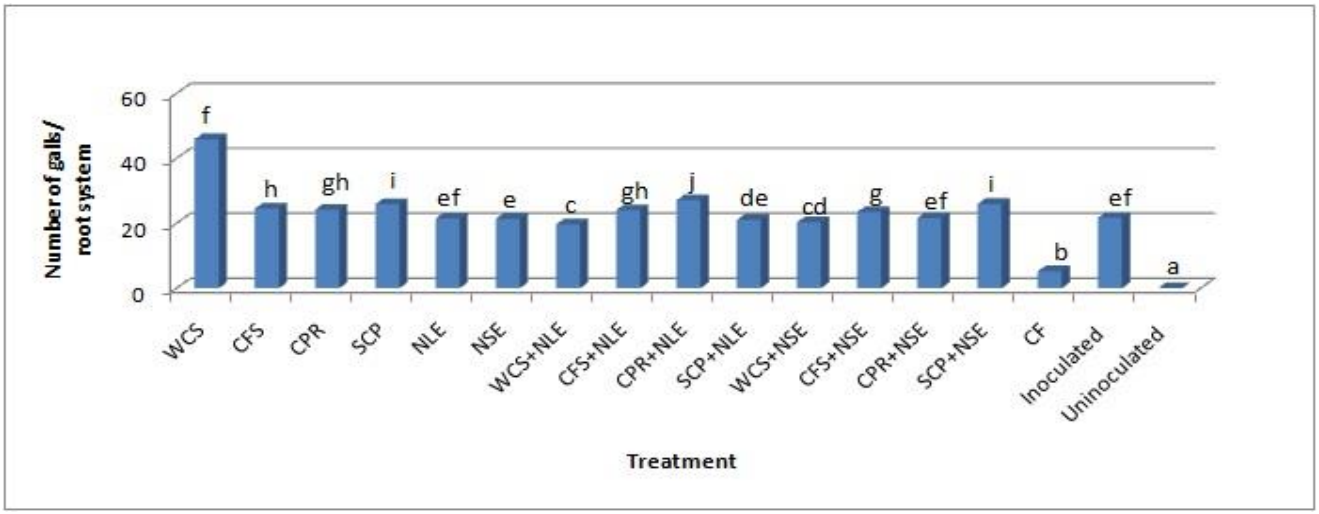

Figure 1

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 2

Showing effect of seed dressing treatment with various formulations of neem, Bt and combination of neem + Bt on percentage reduction in the number of J2/1g root of 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	203.20± 3.9	31.6
Neem seed extract	222.91± 3.4	25
Whole cell suspension	202.84± 4.7	31.9
Cell free supernatant	252.46± 3.6	15.1
Cell pelleted residues	226.06± 2.6	23.9
Spore/ crystal proteins	251.64± 3.5	15.4
Whole cell suspension + neem leaf extract	180.91± 2.0	39.3
Cell free supernatant + neem leaf extract	204.5± 3.9	31.3
Cell pelleted residues + neem leaf extract	204.64± 4.0	31.3
Spore/crystal proteins + neem leaf extract	238.05± 3.0	19.8
Whole cell suspension + neem seed extract	251.50± 2.8	15.4
Cell free supernatant + neem seed extract	250.86± 2.3	15.8
Cell pelleted residues + neem seed extract	203.58± 4.2	31.6
Spore/crystal proteins + neem seed extract	251.96± 3.4	15.4
Carbofuradan 3-G	39.25± 4.3	86.9
Inoculated control 1 (C-1)	297.38± 39.4	297.3804 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

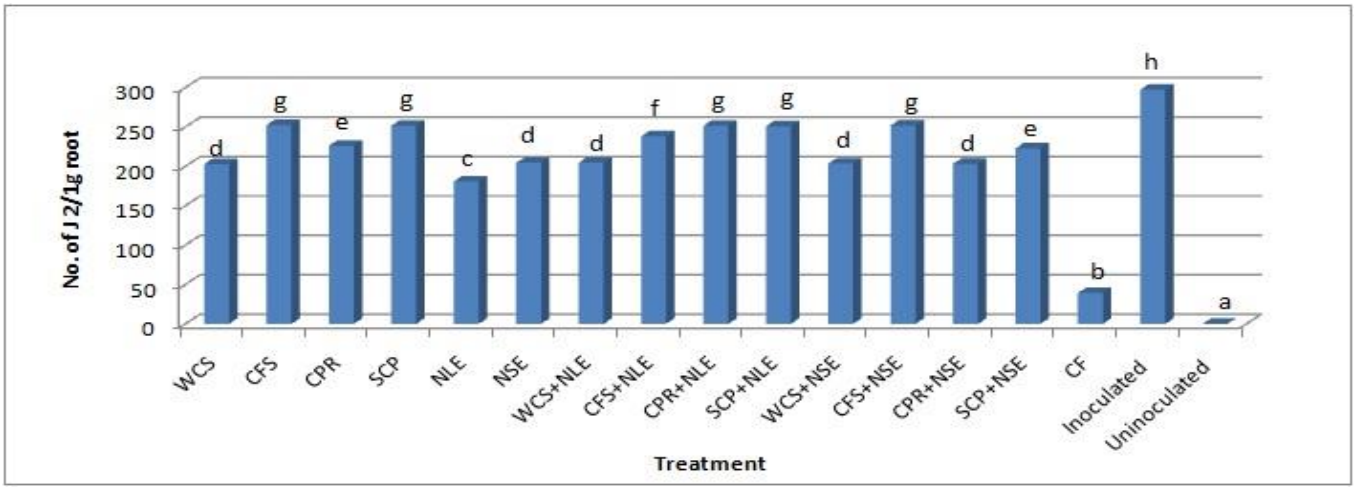

Figure 2

Showing the nematicidal effect of seed dressing 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

Kaur et al.
Nematicidal effect of neem and Bt on *Meloidogyne incognita* infesting tomato plant by seed dressing treatment,
Discovery Agriculture, 2014, 2(6), 28-40,

Table 3

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

Treatments	Number of J2/250g soil	
	Mean	Reduction % age
Neem leaf extract	21.20±2.4	23.7
Neem seed extract	24.27±3.1	12.9
Whole cell suspension	20.63±1.8	25.8
Cell free supernatant	25.18±2.7	9.7
Cell pelleted residues	27.33±2.6	1.7
Spore/ crystal proteins	26.30±2.3	5.3
Whole cell suspension + neem leaf extract	20.18±2.1	27.6
Cell free supernatant + neem leaf extract	20.35±2.0	26.9
Cell pelleted residues + neem leaf extract	20.49±1.4	26.6
Spore/crystal proteins + neem leaf extract	25.41±2.3	8.6
Whole cell suspension + neem seed extract	21.08±1.8	24.4
Cell free supernatant + neem seed extract	21.38±2.7	23.3
Cell pelleted residues + neem seed extract	20.94±1.6	24.8
Spore/crystal proteins + neem seed extract	24.80±2.7	10.7
Carbofuradan 3-G	0±0	100
Inoculated control 1(C-1)	27.81± 2.5	27.81522 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

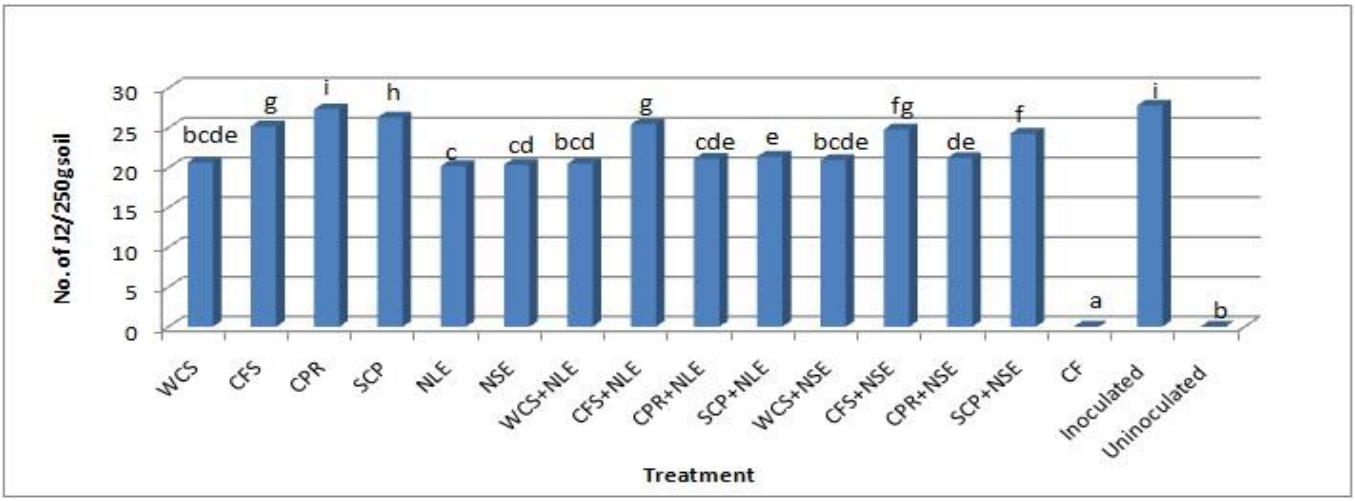

Figure 3

Showing the nematicidal effect of seed dressing 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

Kaur et al.
Nematicidal effect of neem and Bt on *Meloidogyne incognita* infesting tomato plant by seed dressing treatment,
Discovery Agriculture, 2014, 2(6), 28-40,

Table 4

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

Treatments	Number of males/250g soil		
	Mean	Increase %age	Reduction % age
Neem leaf extract	26.22± 2.3	13.4	-
Neem seed extract	31.46± 2.0	35.9	-
Whole cell suspension	21.77± 1.8	-	6
Cell free supernatant	21.64± 2.4	-	6.4
Cell pelleted residues	26.41± 2.5	14.2	-
Spore/ crystal proteins	23.85± 3.8	3	-
Whole cell suspension + neem leaf extract	24.12± 2.2	4.3	-
Cell free supernatant + neem leaf extract	23.28± 2.8	0.4	-
Cell pelleted residues + neem leaf extract	21.11± 2.0	-	8.6
Spore/crystal proteins + neem leaf extract	25.98± 2.4	12.1	-
Whole cell suspension + neem seed extract	26.22± 2.2	13.4	-
Cell free supernatant + neem seed extract	26.78± 2.1	15.5	-
Cell pelleted residues + neem seed extract	20.94± 1.5	-	9.5
Spore/crystal proteins + neem seed extract	25.48± 2.7	9.9	-
Carbofuradan 3-G	13.34± 1.2	-	42.2
Inoculated control 1 (C-1)	23.11± 4.0	23.11413	23.11413
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

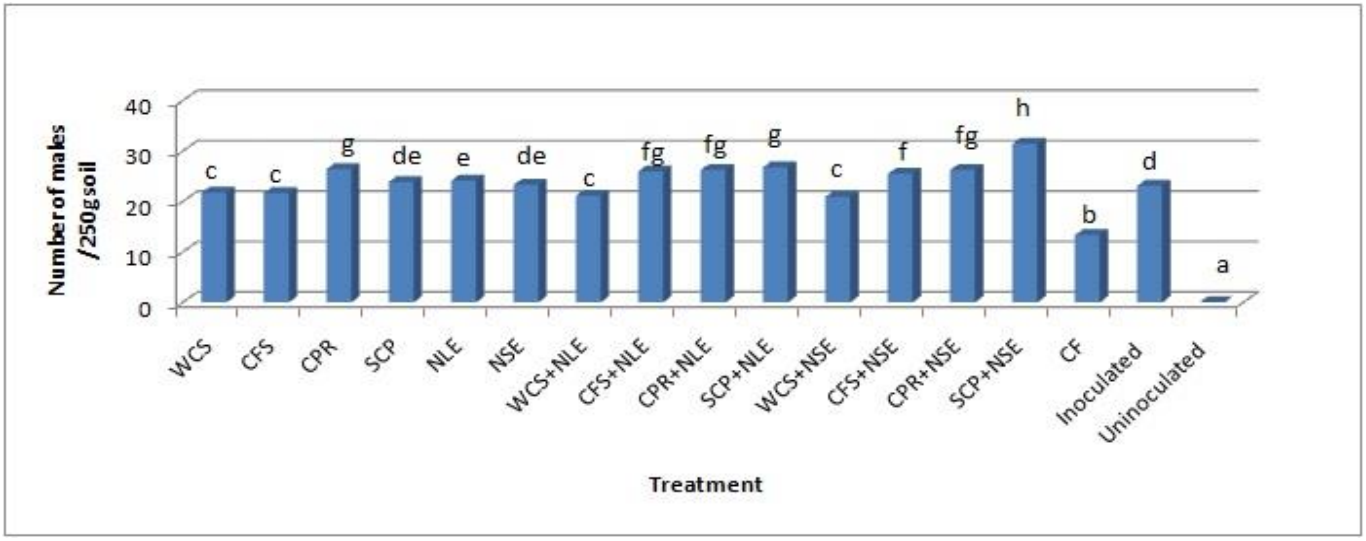

Figure 4

Showing the nematicidal effect of seed dressing 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

Kaur et al.
Nematicidal effect of neem and Bt on *Meloidogyne incognita* infesting tomato plant by seed dressing treatment,
Discovery Agriculture, 2014, 2(6), 28-40,

Table 5

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

Treatments	Number of egg masses/gall	
	Mean	Reduction % age
Neem leaf extract	9.30± 1.5	7
Neem seed extract	8.54± 1.3	15
Whole cell suspension	7.44± 0.9	26
Cell free supernatant	6.84± 1.0	32
Cell pelleted residues	7.77± 0.9	23
Spore/ crystal proteins	9.15± 1.3	8.5
Whole cell suspension + neem leaf extract	6.83± 1.1	32
Cell free supernatant + neem leaf extract	7.09± 0.7	30
Cell pelleted residues + neem leaf extract	7.55± 1.0	25
Spore/crystal proteins + neem leaf extract	8.85± 1.9	12
Whole cell suspension + neem seed extract	6.85± 0.7	32
Cell free supernatant + neem seed extract	7.20± 0.7	28
Cell pelleted residues + neem seed extract	9.22± 1.6	8
Spore/crystal proteins + neem seed extract	10.06± 2.3	0
Carbofuradan 3-G	3.02± 0.7	70
Inoculated control 1 (C-1)	10.01± 0.9	10.0163 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

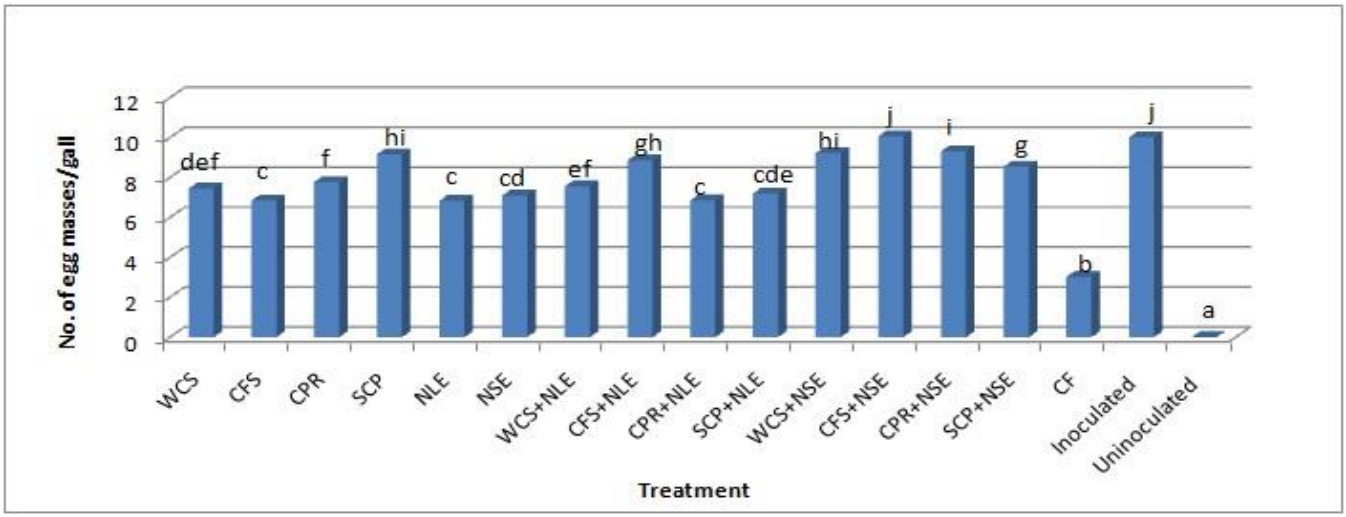

Figure 5

Showing the nematicidal effect of seed dressing 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

Kaur et al.
Nematicidal effect of neem and Bt on *Meloidogyne incognita* infesting tomato plant by seed dressing treatment,
Discovery Agriculture, 2014, 2(6), 28-40,

Table 6

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

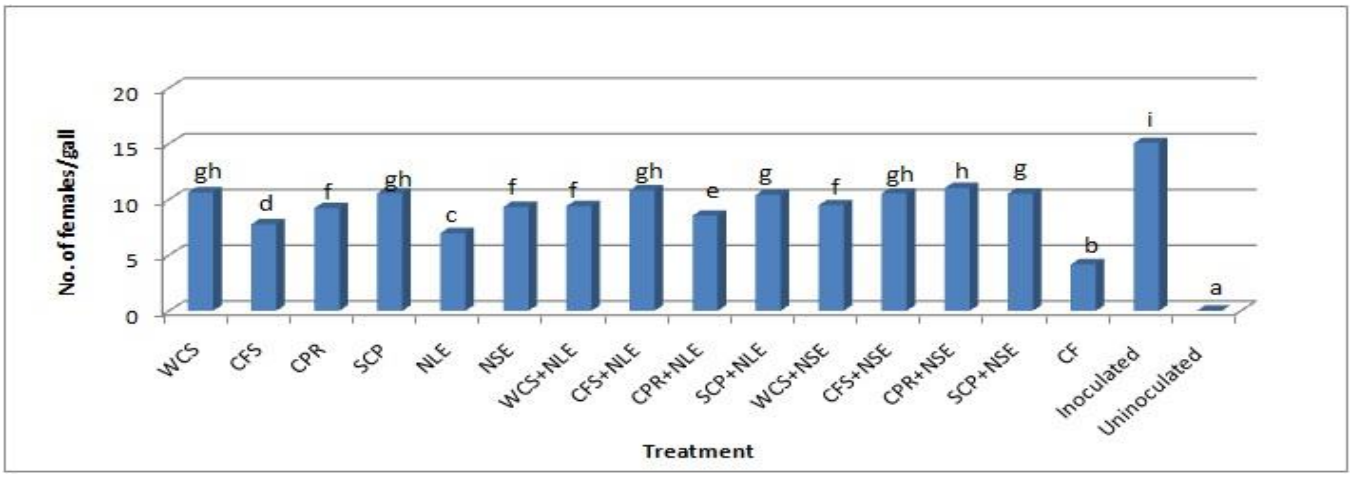

Figure 6

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

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 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

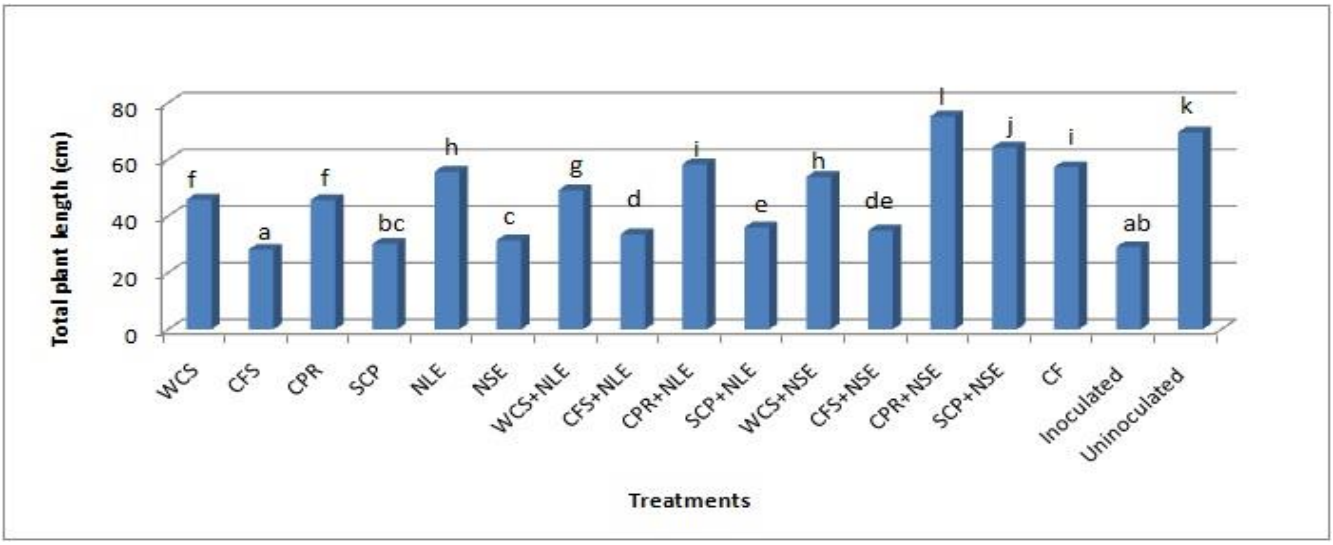

Figure 7

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

Kaur et al.
Nematicidal effect of neem and Bt on *Meloidogyne incognita* infesting tomato plant by seed dressing treatment, Discovery Agriculture, 2014, 2(6), 28-40,

Table 8

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	-
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

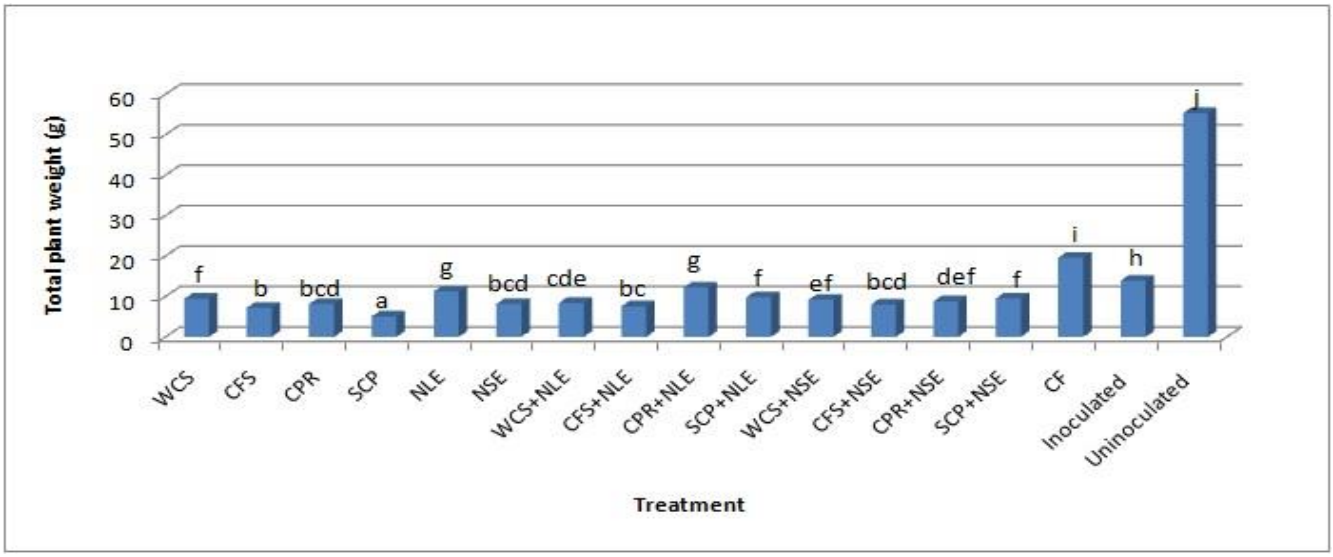

Figure 8

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

Kaur et al.
Nematicidal effect of neem and Bt on *Meloidogyne incognita* infesting tomato plant by seed dressing treatment,
Discovery Agriculture, 2014, 2(6), 28-40,