Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(4): 943-946

Received: 27-05-2019
Accepted: 28-06-2019

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Outcome of pre-sowing nursery treatments on the nematode population, tomato crop status and fruit yield growing in mid-hill area of Himachal Pradesh

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Abstract
Tomato is one of the most important vegetable crop commercially grown in mid-hill region of the state of Himachal Pradesh and comprise the commercial cash crop for the vegetable growers of this area. This crop is responsible for providing economic strength to the commercial growers of district. Unfortunately, tomato is highly susceptible to number of nematode pests, of which *Meloidogyne* ranks at the top. It is often referred as a universal host of *Meloidogyne* species. Looking into the ecological and environmental concerns regarding the use of chemical nematicides, efforts were made to manage the test nematode using integrated approach wherein the use of chemical nematicides were minimised. Treatments with inorganic soil fumigants *viz*., Dazomet, formalin, STTC, carbofuran and seed treatment with fungal formulation of *Pseudomonas fluorescens*, all at recommended doses were separately used in nursery plots against *M. incognita* for raising tomato crop. All the disinfectants were effective against the nematodes as evident from the healthy ungalled roots of seedlings grown in soil treated with any of the fumigants. Maximum germination of 93.6 per cent was observed in Dazomet treated plots followed by 84.0 per cent in plots treated with formalin. Germination in other treatments was appreciably low at 61.0, 59.6 and 49.3 per cent in respective treatments of *P. fluorescens*, carbofuran and STTC. Restricted aerial growth was observed in seedlings growing in STTC treated soil. The best seedling growth was recorded in Dazomet (14.4 cm) and formalin (13.9 cm) treated plots, both statistically at par, in the year 2016, scenario was vice-versa in the year 2017 when seedling length of 14.5 cm attained in the soil treated with formalin was statistically more than 13.0 cm attained in soil treated with Dazomet.

Keywords: Dazomet, STTC, Carbofuran, *Pseudomonas fluorescens*

Introduction
The genus Solanum of the family Solanaceae is believed to have originated in the coastal strip of western South America from the equator to about latitude 30° South [1]. Tomato is one of the most important vegetable crops grown worldwide in an area of more than 5 X 10^6 hectares with the production of approximately 161 X 10^6 MT. The continents of Africa and Asia account for more than 80 per cent of global area under tomato with about 70 per cent of world output [2]. In India, the crop occupies an area of 9930 hectares, yielding 413710 MT of fruits [3]. The major Tomato producing States in the country are Andhra Pradesh, Madhya Pradesh, Karnataka, Gujarat, Odisha, West Bengal, Chhattisgarh, Maharashtra, Bihar, Haryana, Uttar Pradesh, Telangana and Tamil Nadu. Major Tomato producing states in the country are Andhra Pradesh, Madhya Pradesh, Karnataka, Gujarat, Odisha, West Bengal, Chhattisgarh, Bihar, Haryana, Uttar Pradesh, Maharashtra, Tamil Nadu and Telangana [4]. These states are account for 90% of the total production of the country. In Himachal Pradesh, tomato is extensively cultivated in Solan, Shimla and Kullu districts (http://agritech.tnau.ac.in). The mid-hill region of Solan producing 195,900 MT ranks first as far as area under tomato cultivation (4298 hectares) in the state is concerned. Tomato is highly susceptible to all the four major species of root-knot nematode *viz*., *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla*. Referring to the extreme susceptibility of tomato [5] it was reported that *Meloidogyne* spp. had the potential to cause (25-100 per cent) yield losses to this crop. However, under protected conditions the crop suffers significant economic damage to the extent that many farmers have been forced to abandon cropping in polyhouses in the region.

Materials & Methods

1. **Selection of nursery:** The plots already infested with root-knot nematode (*Meloidogyne incognita*), but otherwise suitable for nursery site, were selected for raising of tomato nursery in the experimental farm. The selected nursery site was thoroughly ploughed and beds of ½m^2 size were prepared for test crop.
2. **Sample collection:** Initial nematode population per plot was ascertained by analyzing soil samples collected from each replication. Samples (200 cc soil) were washed using Cobb's sieving and decanting technique [6] followed by Schindler's modification [7] for nematode extraction. Nematode incidence in nursery was recorded by observing one ml of the soil suspension from each sample under the stereozoom microscope and counting of the nematodes. The average of three readings per replicate was used to calculate the nematode population. Number of nematodes in one ml multiplied by amount of suspension gives the nematode count in 200 cc soil. Pre-sowing nursery treatments with various soil disinfectants at recommended doses as given in the following table were applied at random. Each treatment was replicated three times.

Table 1: Pre-sowing nursery treatments with soil disinfectants and seed treatment with *Pseudomonas fluorescens*

Treatment	Composition of soil disinfectant	Formulation used	Rate of application
T_1	Formaldehyde 5 per cent	Formalin	14ml/ ltr
T_2	Tertrahydro- 3,5- dimethyl -1,3,5- thiadiazine -2- thione (Dazomet 98 G)	Sumid	40gm/ m^2
T_3	Sodium tetra thiocarbonate (STTC) 40S	Suzone	15ml/ m^2
T_4	Carbofuran 3G	Furadan	9gm/ m^2
T_5	*Pseudomonas fluorescens*		10g/ kg seed
C	Untreated Control	-	-

Results & Discussion

The nematode infested nursery site was selected and its soil was analyzed for occurrence of plant parasitic nematodes. Though, various plant parasitic nematodes belonging to different genera were observed in the nursery soil, it was *M. incognita* that dominated the scenario with an average nematode count of more than one juvenile per gram of soil. Other nematodes found in low counts were *Helicotylenchus dihystera*, *Tylenchorhynchus mashoodi* and *Macroposthonia* species. A few individuals of *Pratylenchus* and *Xiphinema* species were also observed. However, the population of none of these was sufficient enough to cause any appreciable damage. The overall scenario was indicative of prevalence of *M. incognita* only beyond the threshold level. Thus, *M. incognita* was selected as a test nematode to be managed in nursery.

Pre-sowing nematode population in nursery soil

The pre-sowing nematode count in nursery soil for the years 2016 and 2017 have been presented in Table 2. As evident from given data, soil population of *M. incognita* was found to be above threshold level of one juvenile/g of soil in both the years under reference. Other plant parasitic nematodes occurred merely as contaminants as their population was so low that it did not seem to have any significant effect on seedling growth of test plant. Thus, further observations were confined to the role of *M. incognita* only, on seedling growth of tomato. Six treatments as referred in material and methods were randomly applied, fifteen days prior to sowing of seeds. The plots applied with formalin were covered with transparent polyethylene sheet immediately after treatment. The sheet was removed after a week and deep ploughing of the soil was done to remove the harmful fumes. Waiting period of another eight days was given to remove any residual effect. Thereafter, seeds of tomato cv. Solan Lalima were sown at prescribed spacing. *Pseudomonas fluorescens* was used as seed treatment.

Effect of nursery treatments on per cent seed germination of tomato

The effect of referred treatments on seed germination was visualized at thirty days of sowing. The data generated on this aspect has been detailed here under in Table 3. Per cent germination improved significantly in all the treatments as compared to control in both the years under experimentation. Maximum germination of 93.6 per cent was observed in dazomet treated plots followed by 84.0 per cent in plots treated with formalin in year 2016. The per cent germination in these two treatments differed significantly from each other. Despite, significant improvement in seed germination over control, germination in other treatments was appreciably low at 61.0 59.6 and 49.3 per cent in respective treatments of *P. fluorescens*, carbofuran and STTC; the former two values significantly at par with each other, during this year. Scenario was slightly different in the year 2017 when per cent germination of seedlings in formalin and dazomet applied treatments was statistically similar at 94.3 and 87.6 per cent respectively. Results in other treatments during the year 2017 were similar to that recorded in 2016. Pooling of data of two years revealed highest and statistically at par germination in dazomet (90.6 per cent) and formalin (89.1 per cent) applied treatments. Per cent germination in other treatments *viz*., *P. fluorescence*, carbofuran and STTC was significantly lower at 62.0, 60.8 and 46.8, respectively. As per pooled information, STTC was found to adversely affect the germination as the plots applied with this chemical showed germination (46.8 per cent) statistically as poor as recorded in nematode infected untreated control.

Effect of nursery treatments on the seedling length of tomato

The seedling status in different treatments was assessed in terms of seedling length at the time of transplanting. Ten seedlings were selected at random from each plot and were measured to work out the average length per replication. The information gathered has been presented in Table 4. While the best seedling growth was recorded in dazomet (14.4 cm) and formalin (13.9 cm) treated plots, both statistically at par, in the year 2016, scenario was slightly different in the year 2017 when seedling length of 14.5 cm attained in the soil treated with formalin was statistically more than 13.0 cm attained in soil treated with dazomet. However, both these treatments were assessed to be the best and statistically similar in pooled analysis as far as their effect on seedling growth was concerned. Seedling growth in *Pseudomonas* treated seeds (9.5 and 10.5 cm during the two years) was significantly higher as compared to that attained in STTC and carbofuran treated soil where the respective average length of 5.1 and 7.1 cm were achieved. Seedling growth with an average of 4.9 cm (pooled value) remained significantly poor in the untreated nematode infected control. Studies [8, 9, 10] also revealed dazomet and metam sodium as highly effective disinfectants against *Meloidogyne* sp., when applied to the moist nursery soil. However, Tamara [11] reported the effectiveness of STTC as pre-plant and post-plant fumigant nematicide.

As evident from figures placed in Table 5, healthy, gall free seedlings grew when the seeds were sown in formalin, dazomet and *Pseudomonas* treated plots, thus, indicating high level of efficacy of referred chemicals/bioagent against the test nematode under nursery conditions. These results are in accordance with the results of various scientists who reported high efficacy of dazomet [12] and metham sodium [13, 14] as soil fumigants against root-knot nematode.

The research conducted earlier [15] yielded similar results as during the present studies, when he found reduced nematode galling and enhanced plant growth in tomato plants when the seeds were treated *with P. fluorescens* @ 10g/kg of the seed. The high efficacy of strains of *Pseudomonas* spp. revealed against root-knot nematode infecting tomato plants, which when applied, not only improved the seed germination but significantly enhanced shoot and root weight [16]. Treatments with *P. fluorescens* caused 45 per cent mortality of *M. incognita* juveniles [17] and it was also known to increase the height of tobacco seedling in pot culture [18]. Application of biocontrol agents like *P. fluorescens* not only has lethal effect on nematode, but also enhances the plant growth, supplying many nutritional elements and induction the systemic resistance in plants. Multiplication rate of nematodes has been revealed to be less in the presence of *P. fluorescens* [19].

Surprisingly, slight galling to the tune of 1.5 on an average was observed in seedlings grown in carbofuran treated plots, which is the only recommended nematicide against test nematode in recent times. The reason could be either improper dissemination of the nematicide or development of resistance in the population of nematode used during this experiment. Extent of galling in the seedling roots of referred treatment was significantly at par with those grown in STTC applied and untreated soils, both showing RKN index of 1.8. Earlier [20] reported improved germination, seedling vigour, reduced gall index and increased yield when carbofuran was used in nursery beds for management of root-knot nematode (*Meloidogyne incognita*).

Dazomet when comes in contact with moist soil surface, it breaks down in methyl isothiocynate, formaldehyde, methyl amine and hydrogen sulphide; all these ingredients having nematicidal principles and good penetrability into soil up to 20-30 cm depth where most of the nematodes infecting annual crops thrive. Formaldehyde on the other hand inactivates microorganism by alkylating the amino and sulfhydryl groups of proteins and ring nitrogen atoms of purine bases, thus, acting as a high level disinfectant. Previously [21] reported formaldehyde as an effective treatment against soil borne pathogens including nematodes. However, being expensive and cumbersome to incorporate into the soil, fumigation with these soil disinfectants should either be confined to nursery beds or these should be applied in field/polyhouse only in case of crops of high economic value facing serious soil borne pest problems which cannot be controlled by any other method. Also, this soil disinfectant is yet to find a place in CIB list for being used against soil borne phytoparasitic nematodes.

Table 2: Pre-sowing nematode population in nursery soil

Plot No.	**2016**						**2017**					
	***Nematode population per 200cc soil**						***Nematode population per 200 cc soil**					
	A	**B**	**C**	**D**	**E**	**F**	**A**	**B**	**C**	**D**	**E**	**F**
1,7,13	203.0	15.0	2.0	0	0	4.0	232.6	7.3	9.0	0	1.0	0
2,8,14	227.3	6.0	13.6	2.0	0	0	291.6	0	0	6.7	0	2.0
3,9,15	259.0	8.0	0	14	3.0	1.3	352.6	19.0	0	3.0	0	0
4,10,16	332.0	32.0	8.0	6.0	8.0	0	246.0	0	14.0	0	4.0	0
5,11,17	210.6	0	16.0	2.0	0	0	306.6	16.0	6.3	0	0	0
6,12,18	292.6	10.0	9.0	0	0	0	277.0	1.0	0	0	3.0	0
Average population	254.0	11.8	8.1	4.0	1.8	0.8	284.4	7.2	4.8	1.6	1.3	0.3

*Average of population of three randomly selected plots

A – *M. incognita*; B- *Helicotylenchus dihystera*; C – *Tylenchorhynchus mashoodi*; D – *Macroposthonia* sp.; E- *Pratylenchus* sp.; F- *Xiphinema* sp.

Table 3: Effect of nursery treatments on per cent seed germination of tomato

Treatments	***Percent seed germination in 2016**	***Percent seed germination in 2017**	**Pooled value**
T_1 formalin	84.0 (9.2)	94.3 (9.7)	89.1 (9.4)
T_2Dazomet	93.6 (9.7)	87.6 (9.4)	90.6 (9.5)
T_3 STTC	49.3 (7.0)	44.3 (6.7)	46.8 (6.9)
T_4 Carbofuran	59.6 (7.7)	62.0 (7.9)	60.8 (7.8)
T_5 *Psuedomonas*	61.0 (7.8)	63.0 (7.9)	62.0 (7.9)
T_6 Control	42.3 (6.5)	42.0 (6.5)	42.1 (6.5)
$CD_{0.05}$	0.39	0.61	0.50

*Average of three replications

Figure in the parentheses are $\sqrt{n+1}$ transformed values

Table 4: Effect of nursery treatments on the seedling length of tomato

Treatments	*Seedling length (cm) in 2016	*Seedling length (cm) in 2017	Pooled value
T_1 formalin	13.9 (3.8)	14.5 (3.9)	14.2 (3.9)
T_2 Dazomet	14.4 (3.9)	13.0 (3.7)	13.7 (3.8)
T_3 STTC	4.8 (2.4)	5.4 (2.5)	5.1 (2.4)
T_4 Carbofuran	6.5 (2.7)	7.7 (2.9)	7.1 (2.8)
T_5 *Psuedomonas*	9.5 (3.2)	10.5 (3.3)	10.0 (3.3)
T_6 Control	4.4 (2.3)	5.4 (2.5)	4.9 (2.4)
$CD_{0.05}$	0.26	0.19	0.23

*Average of three replications

Figure in the parentheses are $\sqrt{n+1}$ transformed values

Table 5: Effect of nursery treatments on the Root Gall Index of tomato seedlings

Treatments	*Root Gall Index (1-5) scale #in 2016	*Root Gall Index (1-5) scale in 2017	Pooled value
T_1 formalin	1.0	1.0	1.0
T_2 Dazomet	1.0	1.0	1.0
T_3 STTC	2.0	1.6	1.8
T_4 Carbofuran	1.6	1.3	1.5
T_5 *Psuedomonas*	1.0	1.0	1.0
T_6 Control	1.6	2.0	1.8
$CD_{0.05}$	0.63	0.57	0.48

*Average of three replications

#Root Gall Index on 1-5 scale: 1=No galls, 2=1-10 galls, 3=11-30 galls, 4=31-100galls and 5=100 galls and above

Conclusions

Treatments with inorganic soil fumigants *viz*., dazomet, formalin, STTC and carbofuran and seed treatment with fungal formulation of *Pseudomonas fluorescens*, all at recommended doses were separately used in nursery plots against *M. incognita* for raising tomato seedlings. All the disinfectants were effective against the nematodes as evident from the healthy ungalled roots of seedlings grown in soil treated with any of the fumigants. However, restricted aerial growth was observed in seedlings growing in STTC treated soil. Best plant status was attained in the seedlings raised in dazomet and formalin treated soil closely followed by those raised in carbofuran applied soils and those raised from the seeds treated with *P. fluorescens*.

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