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Hema
Dr. YS Parmar University of Horticulture & Forestry, Nauni, Solan, Himachal Pradesh, India

Anju Shdhakar Khanna
Dr. YS Parmar University of Horticulture & Forestry, Nauni, Solan, Himachal Pradesh, India

Correspondence
Hema
Dr. YS Parmar University of Horticulture & Forestry, Nauni, Solan, Himachal Pradesh, India

Effect of presowing nursery treatments on the plant status and nematode population of tomato

Hema and Anju Shdhakar Khanna

Abstract
An investigation was carried out for two consecutive years, 2016 and 2017, to test the efficacy of different pre sowing nursery treatments with two fumigants viz., dazomet (@ 40g/ m^2) and metham sodium (@ 14ml/ m^2), one organic formulation i.e., jeevamrit (@ 10%), and two bioagents viz., *Trichoderma viride* (@ 50g/ m^2) and *Pseudomonas flourescens* (@ 50g/ m^2), along with untreated control, against root knot nematodes (*Meloidogyne incognita*) infesting tomato nursery. The results revealed that maximum percent germination and shoot lengths were of jeevamrit, dazomet and metham sodium followed by *T. viride* and *P. flourescens* treated seedlings, all being better than control. In reference to nematode soil populations and root gall index, dazomet and metham sodium had better efficacy in comparison to *T. viride, P. flourescens*, jeevamrit and control. There was no significant difference in nematode soil population between jeevamrit and control.

Keywords: Presowing nursery treatments, plant status, nematode population, tomato

Introduction
Tomato, (*Solanum lycopersicum* L), a native of Andean region, is one of the world's largest grown vegetable crops occupying an area of 25, 60,696 ha with an annual global production of 5, 30, 32,413 MT. At national level, the crop is cultivated in an area of 7, 60,000 ha, producing 1, 83, 99,000 MT of fruit yield (FAO, 2016). The low and mid hill regions of the state of Himachal Pradesh are suitable for tomato cultivation, where 11,080 ha of land is occupied by this crop, yielding 4,89,960 MT of tomatoes annually (Saxena *et al*., 2017) [2]. The crop is grown extensively in the mid hill area of the state, where its cultivation has factually transformed the economy of the farmers because the tomatoes grown as summer and rainy season crop in these areas make off season vegetable for adjoining northern plain and thus, fetch high renumeration in the market. The credit of largest production in this region goes to district Solan, which is crowned as 'the city of red gold' owing to the bulk production of tomatoes (Wikipedia/Solan).

Unfortunately, the crop is highly susceptible to various biotic stresses including insect pests, nematodes, fungi, bacteria, viruses, etc. Of these, plant parasitic nematodes are the major factors, responsible for huge qualitative and quantitative losses. In all, 62 species belonging to 20 genera of plant parasitic nematodes have been found to be associated with tomato. Among them, the most commonly occurring and highly destructive are root knot nematodes (*Meloidogyne* spp.) to whom, this crop plays a natural host. The most prevalent among the root knot nematodes is *Meloidogyne incognita*, which incurs huge and almost incalculable losses owing to its wide distribution and lack of awareness and facilities regarding its management in many of the developing nations. Yet, the avoidable yield losses due to this nematode alone, in various states of India have been estimated to the tune of 5.3 to 37.4 percent (AICRP, 2018).

Such huge losses necessitate the implementation of management strategies against this pest. Among various nematode management strategies employed, the most successful has been the chemical control because of its fast results and near complete mortality (Wacheira *et al*, 2009). However, the growing awareness regarding the hazardous effects of inorganic molecules on environment and biodiversity has diverted the interest of researchers towards safer and environment friendly management practices. Since, it is not possible to completely eliminate the chemical molecules from the management strategies, the priority in recent times has been to restrict their use to the nurseries, especially in the transplanted crops. In quest to look for the healthier management tools against *M. incognita* in tomato nursery the present investigation was carried out to record the effect of certain organic and bio formulations as compared to the commonly used fumigants in tomato.

Material and methods
The area already infested with root-knot nematode (*M. incognita*), but otherwise suitable for nursery site, was selected for tomato nursery raising for two consecutive years i.e., 2016 and 2017, in the experimental farm of the department of Entomology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan. The selected nursery site was thoroughly ploughed and was divided into 24 beds each of ½ m^2 size. Soil samples (200 cc soil) were collected from four to five parts of each plot and were processed using Cobb's sieving and decanting technique (Cobb, 1918) [5] followed by Schindler's modification (Schindler, 1961) [6] for nematode extraction. Thus, nematode incidence in the nursery was recorded by observing one ml of the soil suspension from each sample under the stereozoom microscope and counting the nematodes. An average of three seedlings per replicate was used to assess the nematode population. Number of nematodes in one ml multiplied by amount of suspension received from processing the soil gave the average nematode count in 200 cc soil.

Preparation of Jeevamrit
A barrel of 250L capacity was filled with 200L of water. In this, 10 kg dung, 5-10 liter of urine of a desi cow, 2 kg jaggery, 2 kg pulse flour and hand-full of farm soil were added. The suspension was stirred clockwise, thrice every day. This barrel was covered with wet gunny bag for fermentation in the shade for 5 to 7 days. This fermented solution i.e., Jeevamrit was used as one of the presowing nursery treatments.

Application of pre sowing nursery treatments
Pre-sowing nursery treatments with various soil disinfectants at recommended doses were applied according to randomized block design with four replications for each treatment.
Dazomet and metham sodium granules were separately applied over the surface of the well irrigated and well ploughed soil (up to 6 inch deep) @ 20g/ bed in randomly selected eight plots. These beds were drenched thoroughly with water after the treatment. The soil was immediately worked in and the surface was sealed with plastic mulch for 14-15 days. After the removal of plastic mulch, the soil was turned frequently for another 15 days for removal of traces of the fumigant. Irrigation was done every fifth day to keep the soil wet until the completion of the waiting period of 30 days.
The mixture of freshly prepared jeevamrit and water @ 1:10 ratio and was drenched completely into the soil of four randomly selected beds. The plots were ploughed and irrigated after every 5-7 days. The beds randomly assigned as T_4 and T_5 were properly irrigated with water mixed with the bio control agents, *T. viride* (Trade name Sanjeevani, International Panaacea Ltd.) @ 50g/ m^2 and *P. fluorescens* (Trade name Phasal Rakshak, International Panaacea Ltd.) @ 50g/m^2. These beds were irrigated properly on the alternate day to maintain the optimum moisture level. The remaining four beds were maintained as control.
The seeds of susceptible variety of *Solanum lycopersicum* hybrid NS816 were sown in all the prepared nursery beds. All cultural practices for the seedlings were adopted and observations regarding percent germination of seeds per bed, seedling length, final nematode populations and root gall index have been discussed hereunder.

Results
The experiment was laid during two consecutive years, 2016 and 2017 at the nursery site having high *M. incognita* infestation (mean population of 229 J2s/ 200 cc of soil). Seeds of nematode susceptible tomato variety NS 816 were sown in nursery beds receiving various treatments, as mentioned in materials and methods. Observations regarding these treatments on seed germination, seedling growth parameters, nematode population and root galling were recorded.

Effect of nursery treatments on seed germination
The data regarding effect of nursery treatments on seed germination as presented in Table 1 revealed minimum percent germination in nematode infested untreated nursery beds, wherein, average seed germination of 76.5 and 71.75 percent was recorded during the years of 2016 and 2017. Seed germination improved statistically in all the treatments w.r.t control. Whereas, in 2016 the best mean percent seed germination of 94.75 and 93.25 was recorded in nursery beds receiving jeevamrit and dazomet treatments respectively, during 2017, the treatments of dazomet, metham sodium and jeevamrit resulted into best and statistically similar seed germination in the range of 90.25 to 93.5 percent. Though, the mean percent seed germination in *T. viride* and *P. flourescens* treated beds improved significantly w.r.t control during both the years of experimentation, it remained significantly lower than the mean germination recorded in dazomet, metham sodium and jeevamrit treated beds. Statistical pooling of the data for two years also produced similar results, wherein jeevamrit, dazomet and metham sodium treatments produced respective best average germination to the level of 94.13, 92.75 and 90.13 percent. This was followed by statistically lower percent rate of germination in, beds receiving *T. viride* and *P. flourescens* treatments in which, averagely 83.5 and 82.13 seeds out of 100 germinated. The seedlings raised in dazomet and metham sodium treated beds were darker dull green with thin stem and lesser vegetative diameter.

Table 1: Effect of nursery treatments on germination of tomato seedlings

S. No.	Treatments*	Percent germination#		
		2016	2017	Pooled
1	Dazomet @ 40g/m^2	93.25 (75.2)	92.25 (74.0)	92.75 (83.7)
2	Metham sodium @ 14ml/m^2	90.00 (72.0)	90.25 (72.6)	90.13 (81.1)
3	Jeevamrit @ 100ml/l	94.75 (76.8)	93.50 (75.3)	94.13 (85.1)
4	*Trichoderma viride* @ 50g/m^2	82.75 (65.5)	84.25 (66.7)	83.50 (74.8)
5	*Pseudomonas flourescens* @ 50g/m^2	81.50 (64.6)	82.75 (65.5)	82.13 (73.6)
6	Control	76.50 (61.0)	71.75 (57.9)	74.13 (66.4)
	$CD_{0.05}$	4.3	5.2	4.8

*Average of four replications
#Average of ten seedlings per replication
**Figures in parentheses are angular transformed values

Effect on seedling length

The stem lengths of ten randomly selected seedlings from each replicate were measured at the time of transplanting and the figures have been placed in Table 2. It is evident from the data that the seedlings raised in nematode infested beds remained stunted and had minimum average height of 10.4 cm, which was significantly lower than the stem lengths attained in the seedlings raised in treated nursery beds. While the average seedling heights of 15.8, 13.0, 14.8, 10.6 and 11.2 cm in dazomet, metham sodium, jeevamrit, *T. viride* and *P. flourescens* treated beds respectively differed significantly from each other during the year 2016, the mean seedling lengths of 16.6 and 16.8 cm in dazomet and jeevamrit treated beds were statistically at par in the year 2017. Data when pooled also showed similar results, wherein, the seedlings raised in dazomet and jeevamrit treated nursery beds were the tallest and significantly similar at respective mean heights of 16.2 and 15.8 cm. This was followed by the average height of 13.7 cm attained in seedlings raised in the beds treated with metham sodium. The seedlings grown in *T. viride* and *P. flourescens* applied beds reached the average heights of 11.8 and 11.6 cm respectively; both values being statistically at par. The data signified the efficacy of all the treatments as far as improvement of seedling length was concerned; dazomet and jeevamrit producing the best results, closely followed by metham sodium. Though, the seedlings raised in *T. viride* and *P. flourescens* treated beds achieved significantly better growth as compared to control, it was statistically lower than all the other treatments.

Table 2: Effect of nursery treatments on length of tomato seedlings

S. No.	Treatments*	Seedling length (cm)#		
		2016	2017	Pooled
1	Dazomet @ 40g/m^2	15.8	16.6	16.2
2	Metham sodium @ 14ml/m^2	13.0	14.5	13.7
3	Jeevamrit @ 100ml/l	14.8	16.8	15.8
4	*Trichoderma viride* @ 50g/m^2	10.6	13.0	11.8
5	*Pseudomonas flourescens* @ 50g/m^2	11.2	12.1	11.6
6	Control	10.0	10.8	10.4
	$CD_{0.05}$	0.4	0.5	0.5

*Average of four replications

#Average of ten seedlings per replication

Effect on nematode population in soil and root gall index

Nematode population and root gall index are crucial factors in assessing the efficacy of adopted control measures. Only those management tools are considered effective, which check the multiplication of RKN, reduce root galling of the roots and improve the plant growth and yield. Statistical analysis of data, referred in Table 3 is indicative of significant difference in J2 population of *M. incognita* in rhizosphere of seedlings grown in untreated and treated nursery beds. Minimum and statistically similar average nematode population of 37.0 juveniles was recorded in the soil fumigated with dazomet and metham sodium. The soil applied with bioagents viz. *T. viride* and *P. flourescens* harboured mean populations of 82.7 and 95.0 individuals, respectively. These counts were significantly similar to each other but differed statistically from the populations recorded in dazomet and metham sodium treated beds. The pooled average nematode population of 221.2 individuals recorded in beds drenched with jeevamrit was significantly similar to the mean population of 289.0 J2s recorded in untreated beds.

Table 3: Effect of nursery treatments on *M. incognita* soil population at 30 days of seed sowing

S. No.	Treatments*	Nematode population (J2s/200cc of soil)#		
		2016	2017	Pooled
1	Dazomet @ 40g/m^2	35.5 (5.8)**	38.5 (5.9)	37.0 (6.1)
2	Metham sodium @ 14ml/m^2	43.5 (6.4)	30.5 (5.1)	37.0 (6.1)
3	Jeevamrit @ 100ml/l	242.5 (15.5)	200.0 (14.0)	221.2 (14.9)
4	*Trichoderma viride* @ 50g/m^2	95.5 (9.7)	70.0 (8.3)	82.7 (9.1)
5	*Pseudomonas flourescens* @ 50g/m^2	108.5 (10.4)	81.5 (9.0)	95.0 (9.7)
6	Control	321.0 (17.9)	257.0 (16.0)	289.0 (17.0)
	$CD_{0.05}$	2.3	3.0	2.6

*Average of four replications

#Average of ten seedlings per replication

**Figures in parentheses are square root transformed values

Indexing of root galls on a designated scale is significant to analyse the pathogenic activity of *M. incognita* in presence and absence of control measures. The figures placed in Table 4 refer to the extent of gall formation due to nematode infestation in different treatments. Mean root gall indices (on 1-5 scale) on roots of untreated seedlings at 2.60 and 2.25 during respective experimental years of 2016 and 2017, were significantly higher than the indices recorded on the roots of treated plants. The only exception was the gall index of 2.00 recorded on the seedling roots raised in jeevamrit drenched soil during 2017, which was statistically at par with control. However, the data when pooled, showed the significant difference between the root gall indices recorded on the roots of seedlings raised in treated and untreated beds. Minimum average root gall index of 1.14 (merely nil to 2 galls per root) was recorded on roots of seedlings raised in dazomet and metham sodium treated beds. The seedlings raised in jeevamrit, *T. viride* and *P. flourescens* applied beds had statistically similar amount of galling, which was significantly lower than that recorded in seedlings raised in untreated beds.

Table 4: Effect of nursery treatments on root galling of tomato seedlings

S. No.	Treatments*	Root gall index (on 1-5 scale)**#		
		2016	2017	Pooled
1	Dazomet @ 40g/m^2	1.15	1.13	1.14
2	Metham sodium @ 14ml/m^2	1.20	1.08	1.14
3	Jeevamrit @ 100ml/l	2.10	2.00	2.05
4	*Trichoderma viride* @ 50g/m^2	1.95	1.65	1.80
5	*Pseudomonas flourescens* @ 50g/m^2	1.90	1.90	1.90
6	Control	2.60	2.25	2.43
	$CD_{0.05}$	0.30	0.3	0.32

*Average of four replications
** Root gall index on 1-5 scale (1= no gall, 2= 1 to 10 galls, 3= 11 to 30 galls, 4= 31 to 100 galls and 5= above 100 galls)
#Average of ten seedlings per replication

Discussions

The results signified the efficacy of various treatments against *M. incognita* when compared with control. Though, the percent seed germination and seedling growth improved in all the treatments, it was jeevamrit application in which the seedlings raised showed highest germination, vigorous and healthy seedling growth, despite the presence of *M. incognita* above ETL. Application of matured organic molecules improve the soil nutrition and act as growth regulators, thus, resulting into enhancement of growth parameters. However, jeevamrit did not check the nematode multiplication significantly. The bioagents, viz., *T. viride* and *P. flourescens* showed significant increase in plant growth parameters and reduced nematode multiplication in the soil but were less effective than the chemical fumigants and jeevamrit. Whereas, the bioagents reduced nematode soil populations better than jeevamrit, seedling growth was significantly lower.

Soil disinfectants viz. dazomet and metham sodium resulted in minimum nematode population after 30 days of seed sowing. Earlier Giannakou and Karpouzas (2003) [7] and Desaeger *et al,* (2004) [8] reported high efficacy of metham sodium and dazomet as soil fumigants against root knot nematode in cucumber. According to Neshev (2008) [9], dazomet and metham sodium when applied to the moist soil were highly effective against nematodes like *Meloidogyne* sp., *Rotylenchus* sp., *Pratylenchus* sp. and *Heterodera* sp. Chandel *at al.* (2014) [10] also reported fumigation with metham sodium as the best option in mitigating effect of root knot nematode. Dazomet when comes in contact with moist soil surface, its active substance breaks down in methyl isothiocynate, formaldehyde, methyl amine and hydrogen sulphide; all these ingredients having nematicidal principles and good penetrability into the soil up to 20-30 cm depth where most of the nematodes infecting annual crops thrive. Similarly, metham sodium in contact with moist soil decomposes into methyl isothiocynate which is again highly effective against *Meloidogyne* spp. However, being expensive and cumbersome to incorporate into the soil, fumigation with these soil disinfectants should be confined to nursery beds.

So far no work has been done on the evaluation of jeevamrit against *M. incognita,* however, a study on effect of cow dung and cow urine, which are the ingredients of jeevamrit, alone and in combination done by Abubakar *et al.* (2004) [11] against *M. incognita* revealed that the tomato plants inoculated with *M. incognita* and treated with the combination of cow dung and urine showed maximum plant height and plant dry weight. They also reported that root gall index was not as low as recorded in the control treatment where the recommended nematicide was used. Pakeerathan *et al.*, (2009) [12] also used various animal waste products against *M. incognita* and concluded that animal manures could be used as an alternative for the management of *M. incognita* and were more or less equally effective as nematicides.

Biocontrol agents like *T. viride* and *P. flourescens* not only check the multiplication of *M. incognita* but also have known positive effects on plant growth parameters due to their facultative saprophytic nature, which is an additional support in soil up gradation. While their efficacy was lesser in comparison to the fumigants, the results were better than that from untreated beds. Muthulakshmi *et al*. (2010) [13] and Nama *et al.* (2013) [14] reported soil application of both *P. fluorescence* and *T. viride* to be effective in checking the population of *M. incognita* and improving the crop yield. Sen *et al.* (2016) [15] also concluded that bio-agents viz. *T. viride, T. harzianum* and *P. fluorescens* were better over untreated check (control) in improving plant growth characters of brinjal and reducing nematode reproduction. Thengal *et al.* (2017) also revealed that *T. viride* brought about a significant reduction in number of galls, egg masses, and final nematode population in soil over control. Whereas, Senapati *et al.* (2016) [17] concluded positive plant growth results and reduction in nematode populations with *T. viride* and *P. fluorescens* soil application in the tomato field.

Conclusion

The inference from the present investigations suggest that the damage potential of *M. incognita* on tomato is very high even in nursery conditions, and visible damage in the form of galls, and depleted growth are evident in untreated seedlings. This damage can be reduced by employing management practices from the nursery level. Also, the highly effective, but otherwise hazardous chemical control can also be restricted to nursery treatments, and better, and healthier seedlings can be obtained thereafter. Fumigants like dazomet and metham sodium that are highly volatile, are best to be used in nursery soil application, as they vaporise and do not affect the seed germination or seedling growth. Organic preparations and biocontrol agents like jeevamrit, *T. viride* and *P. flourescens* can be classified as better plant growth regulators but are less effective nematicides in comparison to chemical control.

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