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Anupam
Department of Plant Pathology, Punjab Agricultural University, Ludhiana, Punjab, India

Narpinderjeet Kaur Dhillon
Department of Plant Pathology, Punjab Agricultural University, Ludhiana, Punjab, India

Sukhjeet Kaur
Department of Vegetable Science, Punjab Agricultural University, Ludhiana, Punjab, India

HS Buttar
Department of Plant Pathology, Punjab Agricultural University, Ludhiana, Punjab, India

Corresponding Author:
Anupam
Department of Plant Pathology, Punjab Agricultural University, Ludhiana, Punjab, India

Efficacy of non-chemical methods for management of root-knot nematode (*Meloidogyne incognita*) in tomato in protected cultivation

Anupam, Narpinderjeet Kaur Dhillon, Sukhjeet Kaur and HS Buttar

Abstract
Protected cultivation technology helps to cope up with the climatic uncertainties and is lately becoming farmer's choice for production of crops like tomato, capsicum and cucumber. Amongst the different pathogens attacking tomato grown under protected structures, root knot nematode is an increasingly becoming a constraint for sustaining productions. Deregistration of frontline nematicides has shifted focus to alternative methods for management of this pathogen. The present studies, therefore intends to evaluate and understand the comparative efficacy of non-chemical methods with chemical methods for management of *Meloidogyne incognita*. Application of Dazomet @ 40g/sq.m showed maximum decrease in root knot nematode infestation in soil as well as roots of tomato. Amongst the non-chemical methods, neem cake and marigold @ 250g/sq.m exhibited decrease in nematode infestation effectively. Shoot length and shoot weight were also increased by application of these treatments giving additive effect. In the present study, though the application of chemicals comparatively decreased nematode infestation to greater extent as compared to non-chemical treatments, but due to their ill effects on health and environment as well as tomato being directly consumable these are not suggested for use. So, the use of eco-compatible non-chemical methods of neem cake as well as marigold are useful option for management of root knot nematode in tomato under protected cultivation.

Keywords: *Meloidogyne incognita*, root-knot nematode, tomato, chemicals, non-chemicals, protected cultivation

Introduction
Tomato (*Solanum lycopersicum* L) is one of the most important commercial and widely grown vegetable crops in both tropics and sub- tropics, which is consumed in various ways. It is grown in open as well as in protected cultivation. In India, tomato is cultivated on 808.54 thousand hectare area with production and productivity of 19696.92 thousand million tonnes and 24.36 million ton/ha respectively during 2016-17 (Anonymous, 2017). In Punjab, tomato is grown on 10.17 thousand hectare area with 252.63 thousand tons of production during year 2019 (Anonymous, 2019) [4]. A number of biotic and abiotic stresses impose constraints to the production of this crop. Plant-parasitic nematodes are important biotic constraint responsible for global agricultural losses amounting to an estimated $157 billions annually (Abad *et al.*, 2008) [1]. Among these plant parasitic nematodes, the root-knot nematodes (*Meloidogyne* spp.) are most damaging agricultural pests attacking several crops (Sahebani and Hadavi, 2008) [28]. These species have a wide host range encompassing more than 3000 plant species (Ibrahim *et al.*, 2011) [14]. These parasitic nematodes not only cause direct damage to plants, but they can also facilitate infections by other phytopathogens, including those of fungi, bacteria and viruses (Ashraf and Khan, 2010; Greco and Di Vito, 2009) [6, 13]. They are capable of surviving in the soil in the form of eggs or in weeds in the absence of host. Root-knot nematodes are most destructive and difficult to control once established as they persist in the soil for longer period inside the soil (Sikora and Fernandez, 2005) [31]. Researchers all over the world are engaged in standardizing the nematode management strategies by following non-chemical and ecofriendly approaches such as cropping systems soil amendments (botanicals) (Sukul *et al.*, 2001; Rajendran and Saritha, 2005) [32, 26], organic soil amendment (Vedhera *et al.*, 1998) [38], biological control agents (Kantharaju *et al.*, 2005; Sumathi *et al.*, 2006) [19, 33] and judicious use of nematicides (Taylor and Sasser, 1978) [35, 37] to stabilize crop production.
In India, the losses caused in tomato cultivation due to this nematode are very high i.e. 27.21 % and monetary loss was calculated up to 2204 million rupees (Jain *et al.*, 2007) [16].

Plant parasitic nematodes (*Meloidogyne* spp.), are the most destructive and difficult to control in protected cultivation system (Sharma *et al*., 2007). Tomato yield losses of up to 80% in heavily infected soils have been recorded (Kaskavalci, 2007). Severe infestations can kill a tomato plant outright (Kamran *et al*., 2010). Pest and disease assume new proposition in protected cultivation of crops due to moderate climate and intensive cultivation. With this technology, the problems of nematodes had cropped up. Therefore, a fresh look into the dynamics of soil borne pathogens like root-knot nematodes has to be intensified. As the accrued loss due to these pests is tangible, proper attention is must for their management. Various chemicals for the management of nematodes have been used under protected cultivation but due to their health and environmental hazards these are not suggested for use. In view of this, the present study was undertaken to evaluate the effect of different non-chemical options for management of root-knot nematode in tomato cultivation.

Materials and Methods

The present trials were conducted for two years (2016 & 2017) in pot house of Department of Plant Pathology, Punjab Agricultural University, Ludhiana. In all, ten different treatments of chemical and non-chemical methods were taken. Amongst different non-chemical methods, neem cake @ 250g/sq.m, marigold @ 250g/sq.m, poultry manure @ 250g/sq.m, farm yard manure @ 250g/sq.m and *Trichoderma harzianum* @ 1.5kg/sq.m + farm yard manure (125g/sq.m) were taken. In chemical methods, Dazomet @ 40g/sq.m, formalin @ 2% & 3% and carbofuran @0.75g a.i/m^2 were applied along with untreated control. Pots of 15 cm diameter were filled with infested soil with known initial population and these were given treatments as listed above. All the amendments and chemicals (Dazomet and Formalin) were applied ten days before transplanting. These treatments were thoroughly mixed with soil and pots were covered with polythene sheet in formalin treatment to avoid vaporization. Application of carbofuran was done at the time of transplanting. Each treatment was replicated thrice. After removal of sheet, soil was upturned twice for two days and seedlings were transplanted. The seedlings of tomato variety, 'Punjab Ratta' were raised in pot trays containing sterilized cocopeat.

At 3-4 leaf stage, four nursery seedlings were transplanted in each pot. The raising and care of nursery and transplanted plants was done as per Punjab Agricultural University recommendations. After fifty days of transplanting, observations were recorded on root galling index (RGI), soil nematode population and growth parameters. Root galling index was recorded as per the (0-5) scale given by (Taylor and Sasser, 1978) [35, 37] where 0 = no galls; 1 = 1–2; 2 = 3–10; 3 = 11–30; 4 = 31–100; and 5 = more than 100 galls. For root knot nematode population estimation, 250 cc soil samples were taken from individual pot and washed using modified Cobb's sieving and decanting method (Cobb 1918). The reproduction factor (Rf) was calculated as the ratio of final nematode population to initial nematode population (Rf = Pf/Pi), where, Pf= final population; Pi= initial population; Rf > 1 denotes reproduction; Rf < 1 implied no reproduction. In order to record observations on the roots, pots were watered and plants were uprooted gently. The roots of each plant were washed under tap water and spread on a white paper and on the basis of number of galls each plant was graded as given below:

$$\text{RGI} = \frac{\text{Sum total of grades of all the plants observed}}{\text{Total number of plants observed}}$$

The data was subjected to analysis of variance (ANOVA) and CRD for significant differences using CPCS1.

Results and Discussion

The studies conducted for two years (2016 & 2017) on the comparative efficacy of chemical and non-chemical methods against *Meloidogyne incognita* revealed variable effects of different treatments on nematode infestation in soil as well as roots of tomato plants (Table 1).

Effect of chemical and non-chemical methods on *Meloidogyne incognita* in tomato:

Amongst chemical methods, Dazomet @ 40g/sq.m was found to be most effective followed by Formalin @ 3% in managing the root knot nematode infestation. Application of Dazomet (40g/sq.m) and formalin @ 3% showed 79 and 74 per cent reduction in soil nematode population respectively during the first year trial. Very few galls were observed in the pots treated with Dazomet (40g/sq.m) and formalin @ 3% (RGI: 1.33-1.76). The reproduction factor was observed to be < 1 in Dazomet and Formalin treatment. Both these treatments were found to be significantly higher than control in decreasing nematode infestation. The trials conducted with the same treatments in 2017 also showed consistent results as observed in 2016. Dazomet was found to be most effective for management of root knot nematode amongst all the treatments.

Amongst non-chemical methods, during the year 2016, the reduction in nematode infestation in soil amendment with marigold and neem cake @ 250g/sq.m was observed to be more effective than other non-chemical treatments. Soil nematode population was decreased by 68 per cent while root galling index was reduced by 69 per cent in treatment with marigold. Reproduction factor was observed to be < 1 in soil amended with marigold. Application of neem cake @ 250g/sq.m decreased nematode population by 68 per cent and root galling index by 59 per cent. The application of neem cake was found to be advantageous and did not support multiplication of root knot nematode (Rf: 0.84). The treatments of neem cake and marigold were observed to be statistically at par with each other. Comparatively application of Dazomet was observed to be significantly more effective than above treatments. Integrated application of *Trichoderma harzianum* @ 1.5kg/sq.m and farmyard manure @ 125g/sq.m also decreased nematode population significantly in soil (61%) as well as in roots (48%) of tomato. The decrease in nematode population in poultry manure @ 250g/sq.m application was observed to be 42%. The application of farmyard manure @ 250g/sq.m was comparatively not found to be as effective as neem cake and marigold treatment where reproduction factor was observed to be > 1. The trials in 2017 also revealed similar trends of efficacy in different treatments.

The present results indicated that application of Dazomet was most effective in management of root knot nematode infestation (Figure 1). The reduction of nematode infestation by Dazomet was also reported by Indirani and Jayakumar (2006) who conducted the field experiment thrice to study the efficacy of Dazomet and found that Dazomet effectively

controlled the root-knot nematode, *Meloidogyne incognita.* Soil sterilization with formalin also significantly decreased nematode infestation in soil and roots of tomato. The reduction in *Meloidogyne incognita* by formalin on cucumber in polyhouse was observed by Patil *et al* (2018).

Amongst the different amendments, Marigold and neem cake @ 250g/sq.m plays the crucial role in limiting the root-knot infestation in tomato crop. Suppression of nematode population by marigold may be due to allelopathic effect of chemical alpha-terthienyl released from roots, which is one of the most toxic naturally occurring compounds (Bhattacharyya, 2017). This compound is known to possess nematicidal, insecticidal, antiviral and cytotoxic properties (Marles *et al.*, 1992). It caused significant reduction in the number of root galls, root nematode population and soil nematode population with a remarkable growth of tomato plant (Mali *et al.*, 2019). In addition to allelopathic effects, other suggested mechanisms of marigold responsible for nematode suppression include enhanced activity of endophytic bacteria (Sturz and Kimpinski, 2004) and stimulation of nematode-antagonistic organisms (Kimenju *et al*., 2004). The nematicidal effect of marigold root extract could also be due to a combination of several potential allelopathic compounds including biologically active essential oils and not solely α-terthienyl (Franzener *et al.*, 2007). Neem cake @ 250g/sq.m was also found to be effective in managing root knot nematode. Azadirachtin is the main active content of neem and is reported very effective and target specific to controlling insects and nematodes pests of the various crops. It has been reported that certain micro-organisms that contributed to the decomposition of neem cake produce certain products like ammonia, fatty acids, formaldehyde, and phenols (Oka *et al.*, 2000) [24]. The effect of these combined factors leads to reduced nematode development. Our results are supported by the study of Ravishankar & sharma (2005); Ganai, *et al* (2014) [12]; Satyandra *et al* (2011) [29]; Babul & Rana (2012) [7] Archana & Prasad (2014) [5] states that organic amendments of soil using dried poultry litter, municipal refuse, oil cakes of ground nut, neem, mustard & neem products (which are commercial available in market) have been found effective in the control of *Meloidogyne incognita*.

Effect of chemical and non-chemical methods on growth parameters of tomato

The shoot length and shoot weight of tomato plants was significantly increased by application of different chemical and non-chemical treatments as compared to control. The two year trial study revealed that maximum shoot length (62.76cm) and shoot weight (21.57cm) were recorded in neem cake treatment @ 250g/sq.m followed by marigold @ 250g/sq.m. The increase in growth parameters were also observed in Dazomet treatment though their increase was comparatively less than amendment's treatment (Figure 2). The increase in growth parameters in any crop by amendments is due to the fact that additives served as manures (Ahmad *et al*., 2004) [2]. A number of mechanisms have been proposed to explain observed beneficial effects of organic amendments on plant growth in the presence of nematodes. Most reviewers mention release of nematicidal compounds from decomposing materials, stimulation of natural enemies of nematodes, and improved plant growth and tolerance to nematodes (Oka, 2010; Thoden *et al*., 2011) [23, 36]. Incorporation of organic amendments into the soil along with suppressing the nematode population density also promote the antagonistic microbial activity and improve the fertility and organic matter status of the soil (Barani and Anbarani (2004) [8]; Shukla and Tyagi (2009) [30].

Conclusively, the present studies revealed that application of neem cake and use of marigold are viable options for management of root knot nematode. The application of chemicals though effective cannot be advocated due to ill effects to health and environment especially in directly consumable crops like tomato. Further studies need to be conducted for development and standardization of these protocols for their use as bio-formulation in management of root knot nematode under protected cultivation.

Table 1: Efficacy of chemical and non-chemical methods against root-knot nematode in tomato

Treatments	1ST YEAR (2016)					2ND YEAR (2017)				
	Soil nematode population*			Root gall index (RGI)		Soil nematode population*			Root gall index (RGI)	
	Soil population/ 250cc	Per cent Reduction over control	Reproduction Factor* ($Rf=P_f/P_i$)	RGI (0-5) scale	Per cent Reduction over control	Soil population/ 250cc	Per cent Reduction	Reproduction Factor** ($Rf=P_f/P_i$)	RGI (0-5) scale	Per cent Reduction
T_1: Neem cake@ 250g/sq.m	204.30 (14.67)	68.42	0.84	2.03	59.40	183.33 (13.54)	72.12	0.71	2.27	54.60
T_2: Marigold @ 250g/sq.m	202.53 (14.47)	68.70	0.82	1.53	69.40	178.83 (13.54)	72.80	0.69	1.40	72.00
T_3: Poultry manure @ 250g/sq.m	371.76 (19.30)	42.54	1.46	3.03	39.40	258.83 (13.39)	60.64	1.00	2.26	54.80
T_4: Farm yard manure @ 250g/sq.m	456.00 (21.36)	29.53	1.80	3.26	34.80	372.16 (16.04)	43.41	1.45	3.26	34.80
T_5: Dazomet @ 40g/sq.m	132.16 (11.48)	79.57	0.52	1.33	73.40	103.30 (10.19)	84.29	0.40	2.33	55.40
T_6: Formalin @ 2%	368.83 (19.22)	43.00	1.45	3.11	37.80	368.84 (19.49)	43.91	1.43	2.33	55.40
T_7: Formalin @3%	192.16 (13.73)	74.94	0.64	1.76	64.80	181.06 (13.49)	72.47	0.70	1.93	61.40
T_8: *Trichoderma harzianum* (1.5 kg/ m^2)+ Farm yard manure (125g/sq.m)	246.66 (15.47)	61.88	0.97	2.60	48.00	240.00 (13.87)	63.50	0.93	2.50	50.00

T_9: Carbofuran @0.75g a.i/sq.m	233.33 (15.19)	63.94	1.08	2.76	44.80	236.66 (13.55)	64.01	0.92	2.81	43.80
T_{10}: Control	647.10 (25.41)		2.55	5.00		657.70 (25.66)		2.66	5.00	
CD (P=0.05)	1.64			0.96		1.75			1.17	

*Figures in parentheses are square root transformed values; **Initial population of first year trial; 253.30 nem./250cc soil and initial population of second year trial- 256.66 nem./250cc soil

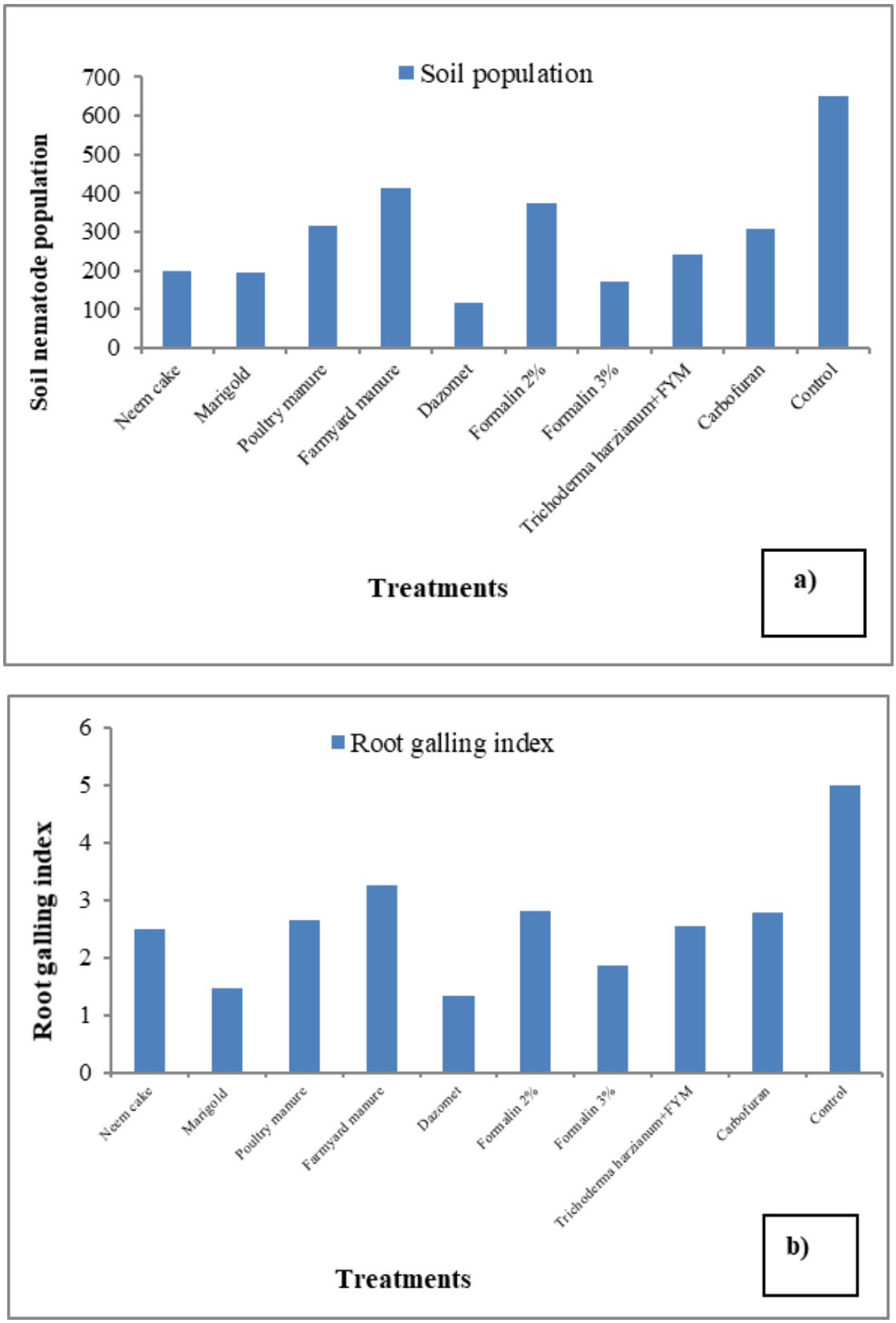
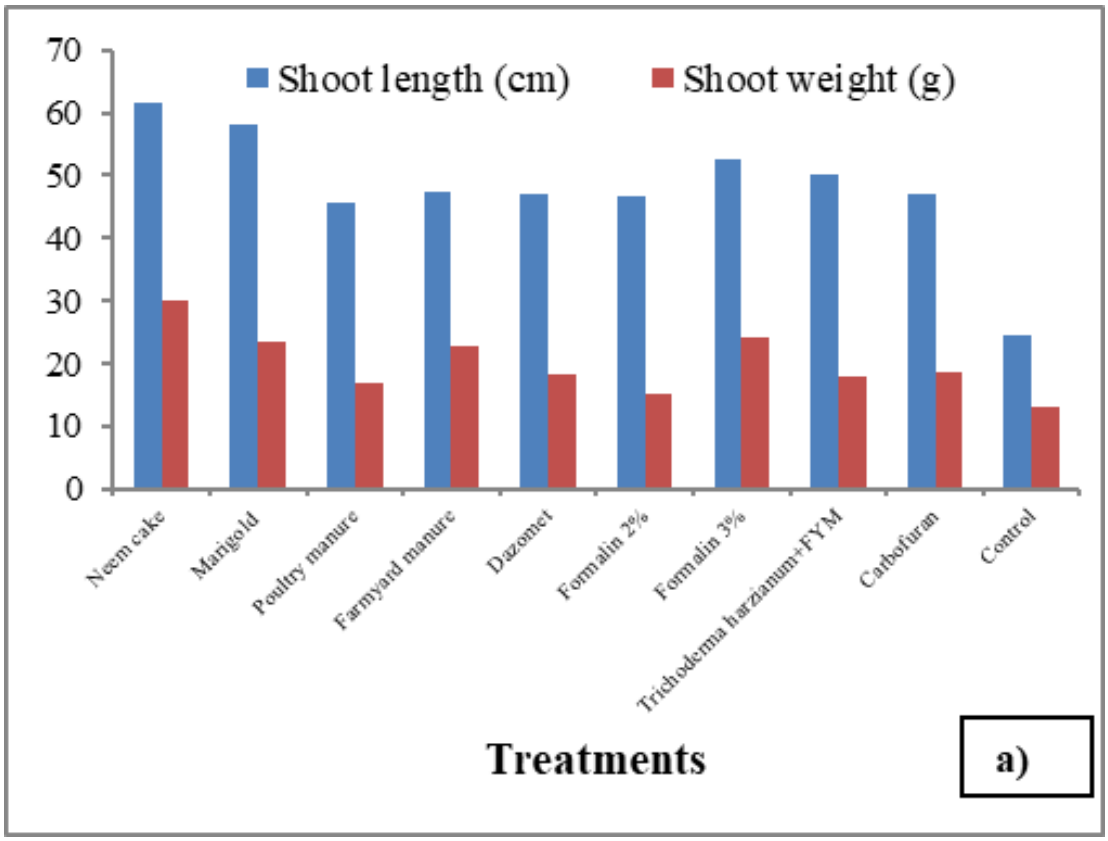


Fig 1: Effect of chemicals and non-chemical methods on a) soil nematode population b) root galling index (Pooled data of two years)



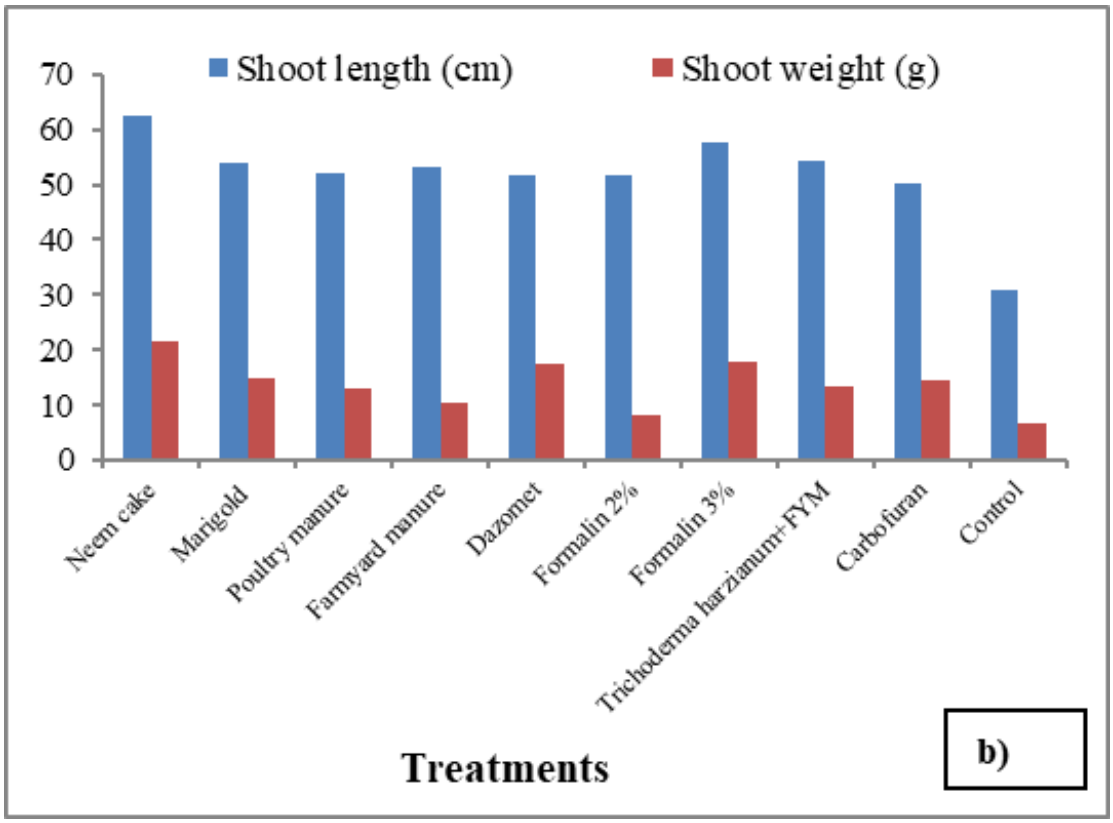


Fig 2: Effect of chemicals and non-chemicals on shoot length and shoot weight a) First year b) Second year

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