MANAGEMENT OF ROOT-KNOT NEMATODES WITH CHEMICAL AND NON-CHEMICAL PESTICIDES OF TOMATO UNDER PROTECTED CULTIVATION

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ABSTRACT

Protected (plastic house) cultivation offers numerous advantages for vegetable production in Nepal. Continuous cropping cycles within soil-based closed structures have led to a rise in soil-borne pathogens, notably in the mid-hills of Nepal, posing challenges to sustainable crop production. Root-knot nematodes (RKNs) particularly threaten protected cultivation, demanding significant time and financial investments for effective management. This study has investigated the efficacy of chemical (fosthiazate) and non-chemical (biogas slurry, *Trichoderma* sp., neem cake, and root care) pesticides with an untreated control for RKN management in tomato grown under plastic houses. A randomized three-block trial was conducted to compare the effectiveness of the treatments. Results revealed significant differences in the galling index (GI), demonstrating that fosthiazate, neem cake, and biogas slurry outperformed the control. In pairwise mean comparison, fosthiazate, neem cake, and biogas slurry were superior to the control with GI of 2.23%, 2.67%, and 3.23%, respectively, where the control had an average GI of 4.45%. There was no significant difference between fosthiazate and neem cake in the galling index, while the performance of *Trichoderma* and root care (a commercial product produced from the combination of organic raw materials) showed no significant difference compared to the control in remediating RKN-induced root galling in tomato. All other treatments gave significantly lower disease incidence than the control. The lowest disease incidence was found to be 12.23 ± (4.10)% and the lowest galling index was 2.23 ± (0.74) % with the application of fosthiazate, followed by neem cake with 16.67 ± (5.56)% disease incidence and 2.67 ± (0.89)% galling index, while the control had the highest disease incidence (80.0 ± (26.67)%. These findings suggest the best management option that may be promising prospects for environment-friendly management of RKN in protected tomato cultivation under plastic houses.

Key words : disease incidence, gall index, *Meloidogyne* spp., plastic house, root-knot nematode management.

INTRODUCTION

Protected cultivation is a new-age farming technique that is highly productive, conserves water and land, and also protects the environment (Jensen, 2002). The growing of crops in plastic greenhouses has reinvigorated farmers' interest in growing off-season or year-round crops for increased economic gains (Kumar et al., 2009). However, the ecological conditions created in protected houses are propitious to certain pests and diseases, which are not frequently confronted by crops grown in open-field cultivation conditions (Sharma et al., 2009). Among the various soil-borne pests, plant parasitic nematodes are becoming a major limiting factor in the sustainable production of tomato crops in protected structures. The most widespread, economically important, and dominant group of plant parasitic nematodes are the root-knot nematodes (Kayani et al., 2012). Root-knot nematodes (RKN) are one of the major pathogens of tomatoes worldwide, limiting fruit production (Sikora and Fernandez, 2005). The use of organic amendments is gaining importance in light of the hazardous nature of the chemicals used for the management of nematode diseases. These amendments are known for their biocidal and antimicrobial activities, as well as their ability to improve soil structure and fertility (Oka, 2010). *Azadirachta indica*—commonly known as neem—is well-documented for its antimicrobial properties (Syndia et al., 2015). Biogas slurry, also known as digestate, is a byproduct of anaerobic digestion that has gained significant attention in recent years as a valuable fertilizer.

The nematicidal effectiveness of *Trichoderma* spp. has been recently proved by both semi-field studies (d'Errico et al., 2021) and field trials (Gautam et al., 2021). *Trichoderma* species are also acknowledged as plant biostimulants, due to the larger availability of water and nutrients related to modified root morphology and rhizosphere interactions (Harman et al., 2004). Root-Care, a commercial product, produced from the combination of organic raw materials, contains various plant extracts, natural organic polymers, chelated micronutrients, and sugars, that affect the root tissues in many ways and activate the biological mechanisms which control the various functions of the root (water and metal uptake, elongation, energy storage and in some cases rebirth). Fosthiazate has been registered as the only nematicide in Nepal to control root-knot nematodes. Upon this background, an experiment was conducted in the vegetable crop development center, in the protected structure (plastic house), to explore the possibilities of employing different chemical and non-chemical management strategies in the control of root-knot nematodes of tomato induced by *Meloidogyne* spp.

MATERIALS AND METHODS

Experimental Site and Design

The experiment was conducted under a protected structure (plastic house) at the field of the Vegetable Crop Development Center, Khumaltar, Lalitpur, Nepal in naturally severely infested soil with *Meloidogyne* spp. dominantly *incognita*, on tomato, variety Srijana, during 2022. Four-week-old tomato seedlings without infestation with *Meliodogyne* spp., verified by microscopic observation, were transplanted into the field. The experiment was laid out in a randomized complete block design with three blocks, and six treatments for three replicates randomized within each block.

Land Preparation, Manuring and Fertilization

The field was ploughed to a fine tilth by giving four ploughings with a sufficient interval between two ploughings. Planking was done for proper leveling. Furrows were then opened at the recommended spacing of 60 cm x 75 cm. Well-decomposed FYM (25 t/ha) was thoroughly incorporated at the time of land preparation. Well-decomposed farm yard manure (FYM) of 63 metric tons, 200 kg of urea, 180 kg of DAP, and 80 kg of MOP per hectare were applied in the field as per the recommendation of the Government of Nepal in Agriculture diary 2081 BS.. The full dose of FYM, phosphorus, potash, and half dose of nitrogen were applied during the field preparation. The remaining half dose of nitrogen was split into two equal halves and top dressed 25 days and 45 days after transplantation as a ring method. The ridges of 30 cm in height and 1 m width were made, and plastic mulch was placed over the ridges after the application of biogas slurry and neem cake.

Treatments and Their Application

There were 6 treatments as: (1) untreated control with nothing applied, (2) soil application of neem cake, (3) soil flooded with 100% biogas slurry, (4) soil treatment with *Trichoderma viride*, (5) seedling treatment with Root Care (hormone), and (6) fosthiazate, a chemical. A greenhouse bioassay was performed to evaluate nematode infection in tomato roots upon application of the above treatments. About 50 kg cow dung from a dairy farm was mixed with 50 L of water and fed to a biogas unit. The bio-digested slurry and biogas were collected through their respective outlets. The slurry was stored in the covered tanks throughout the study period. The biogas slurry was applied at the rate of 50 kg/m^2 to submerge the soil by 5–10 cm thickness of the slurry. The slurry layer was covered with a plastic mulch film for two weeks to increase the soil temperature. Neem cake was applied at a rate of 200 g/ m^2 of soil (Singh et al., 2012). Root Care, a liquid root biostimulant and soil activator, recommended to improve the function of the root system, was applied by spraying the area around the roots three times (at an interval of a month) during the growing season (as specified in the specification of Root Care product), first on the day of transplanting (30th July), second at the time of hoeing and third during the hand pollination period (two months after transplantation), since the experiment was conducted on the tomato intended for seed production at a dosage of 60 ml/m^2 (Wang et al., 2023). Distilled water 196 ml was added into 4 ml potato dextrose broth of *Trichoderma viride* culture to obtain the desired concentration of 10^6 spores/ml and poured into the planting hole (200 ml/hole) just before the plant transplantation (Fan and Wang, 2020). Fosthaizate (active ingredients 30%), 1 ml mixed in 1 L of water drenched the plant @ 500 ml each plant. For effective drug efficacy, plots were irrigated with sufficient water (about 5,000 L/1,000 m^2) within three days of chemical treatment (as specified in the label).

Nursery Raising and Transplantation

Tomato seed, having a germination of 95%, was grown in a cocopeat tray on the 10th of June 2022. Seeds were sown at a depth of 2-3 cm and covered with a fine layer of cocopeat, followed by light watering with a water can. A week before transplantation, the seedlings were hardened by slightly withholding water. The seedlings with 5-6 true leaves were transplanted. In each replication, seedlings were transplanted at a spacing of 60 cm x 75 cm with 12 plants per replication. Not any nematicides were used in soil within one month before the experiment. Thus the results of this experiment were not affected by the previous chemical use.

Irrigation, Intercultural Operation, and Training and Prunning

The field under the plastic house was irrigated through the drip method. Hand hoeing was carried out in the first and third fortnight after transplanting.

Training was done by tying up plants on the wires to prevent lodging and loss of fruit through contact with the soil. Pruning was done with the tipping of the main stem when 3-5 leaves were fully grown, two branches were allowed until 2-4 buds were grown and then again clipped. In this way, four branches were allowed to grow finally.

Identification of *Meloidogyne* species

Meloidogyne spp. were identified by examining the perineal patterns (Jepson, 1987). Adult females were isolated from the infected roots with a fine needle, the anterior part was excised and the posterior part was placed in a solution of 45% lactic acid to remove all body constituents. Perennial pattern was trimmed from the posterior part, transferred to a drop of glycerin, and examined under compound microscope. Six perennial patterns from each treatment representing three blocks were examined.

Disease Assessment and Statistical Analysis

A diagonal five point sampling method was adopted (five plants were selected of a treatment from each replication). The occurrence of root-knot nematode disease in the roots of tomato plants was observed after final harvesting. The root system was rated for galling by a 0 to 5 scale (Anwar et al., 2007), where, 0 = no galls, 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls, and 5 = > 100 galls per root system. The disease incidence, disease index, and control efficacy were computed as follows:

$$\text{Disease incidence \%} = \frac{\text{Number of plants having root knotsymptoms}}{\text{Total plant investigated}}$$

$$\text{Root knot disease index(RKDI)} = \frac{\sum(\text{Number of diseased plants at all levels disease progression})}{\text{Total number of plants investigated} \times 5} \times 100$$

$$\text{Root knot control effect \%} = \frac{\text{Root knot index of control area} - \text{Root knot index of treated area x 100}}{\text{Root knot index of control area}}$$

Data were statistically analyzed using SPSS software 20.0 and SAS (SAS Institute Inc., USA).

RESULTS AND DISCUSSION

Galling Index (GI) of the Treatments

Significant differences in the root knot disease index were found between the treatments, with a p-value of 0.0004. The General linear model (GLM) procedure, which uses the method of least squares, shows that there is high significant difference between the treatments.

On pairwise mean comparison, fosthiazate, neem cake, and biogas slurry were superior to that of the untreated control with root knot disease index of 2.23, 2.67, and 3.23, respectively, where the control had an average root knot disease index of 4.45 (Fig. 1). There was no significant difference between neem cake and fosthiazate in root knot disease index, and the performance of *Trichoderma viride* and Root Care were indifferent to the untreated control for remediation of root-knot nematode-induced root galling in tomatoes.

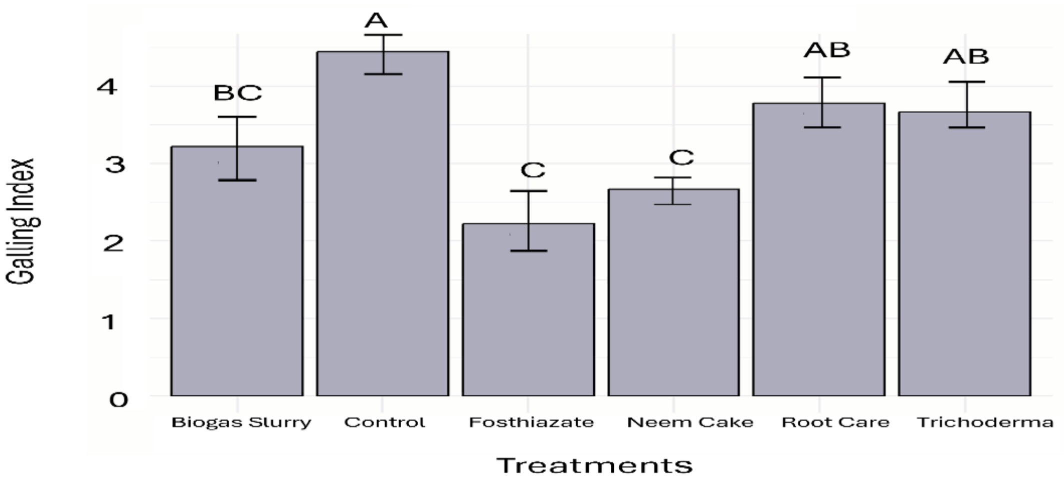

Fig. 1. Error bar chart comparingthe effect treatments on root knot disease index (*Meloidogyne* spp.) in tomato (var. Srijana), Khumaltar, Lalitpur, Nepal, 2022

Assessment of Disease Incidence

Highly significant differences in disease incidence were found between the treatments with a p-value of <0.0001.

On pairwise mean comparison, all other treatments were given significantly lower disease incidence than the control treatment. The mean disease incidence for Control, Root care, *Trichoderma viride*, Biogas Slurry, Neem Cake, and Fosthiazate were 80.0%, 33.34%, 32.23%, 26.67%, 16.67%, and 12.23%, respectively (Fig. 2).

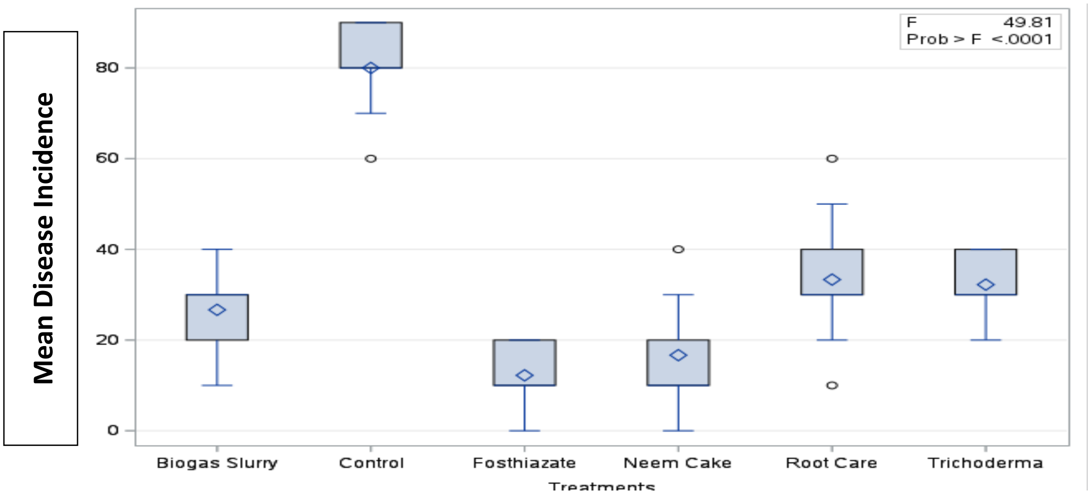

Fig. 2. Boxplot of the root-knot disease incidence percentage in tomato plants (var. Srijana) in various treatments, Khumaltar, Lalitpur, Nepal, 2022

Within each box, square denotes median value; boxes extend from the 25th to the 75th percentile of each group's distribution of values; vertical extending lines denote adjacent values (i.e., the most extreme values within 1.5 interquartile range of the 25th and 75th percentile of each group); dots denote observations outside the range of adjacent value

The lowest mean disease incidence percentage was found to be 12.23 ± (4.10) and the lowest galling index, at 2.23 ± (0.74) with the application of chemical pesticide i.e. fosthiazate. Additionally, neem cake as a non-chemical pesticide has a non-significant difference with fosthiazate at 16.67 ± (5.56), mean disease incidence (2.67 ± (0.89), and root knot disease index (Table 3). However, untreated control has a significant difference in mean disease incidence with 80.0 ± (26.67) and at 4.45 ± (1.48).

Table 1. Effect of treatments on disease incidence and Galling Index of root-knot nematodes on tomato

Treatment	Mean Disease incidence index (Mean ± SE)	Root knot disease index (Mean ± SE)
Fosthiazate	12.23 ± 4.10	(2.23 ± 0.74)
Neem Cake	(16.67 ± 5.56)	(2.67 ± 0.89)
Biogas Slurry	(26.67 ± 8.89)	(3.23 ± 1.10)
Trichoderma viride	(32.23 ± 10.74)	(3.67 ± 1.22)
Root Care	(33.34 ± 11.11)	(3.78 ± 1.26)
Control	(80.0 ± 26.67)	(4.45 ± 1.48)

Similarly, spray of fosthiazate pesticide revealed that 44.44 ± 14.81% root disease index has been graded as the lowest disease index following the neem cake application with 53.33 ± 17.78% disease index. However, Root Care application has the highest disease index (75.56 ± 25.18%) after the control, at 88.89 ± 29.63%. Comparatively, the fosthiazate disease control effect was higher, at 50% than other treatments, followed by neemcake with 40% disease control. *Trichoderma* and Root Care application is found the lowest disease control, at 17.5% and 15% respectively (Table 2).

Table 2. Effect of treatments on root-knot disease index in tomato plants (var. Srijana), Khumaltar, Lalitpur, Nepal, 2022

Treatment	Root disease incidence	Effective control percent
Fosthiazate	44.44 ± 14.81	84.71
Neem Cake	53.33 ± 17.78	79.16
Biogas Slurry	64.44 ± 21.48	66.66
Trichoderma viride	73.33 ± 24.44	59.71
Root Care	75.56 ± 25.18	58.32
Untreated control	88.89 ± 29.63	0

Perineal pattern identification

Microscopic examination of the perineal pattern morphology of adult females from the different fields revealed the presence of three different species, *Meloidogyne incognita*, *M. javanica*, and *M.*

arenaria. Since, the scope of the study was limited with the root knot disease incidence, presence of species was carried without further its distribution and population.

The results of the present study showed that use of more than one treatments of different non-chemical and chemical options is the best management option in reducing the root-knot nematode infestation on tomato in plastic houses. The results obtained are supported by different studies in varied conditions. The process of anaerobic digestion converts organic material into biogas, a sustainable energy source, without the use of oxygen (Şencan et al., 2023). Suppressive effects on *M. incognita* and other RKNs in pots and field application of biogas digestate (BD) have been contrastingly documented but generally acknowledged, suggesting some nematicidal properties of the biogas mixture (Jothi et al., 2003). *Trichoderma* spp. showed less efficiency in the reduction of disease severity in the field, however, it showed good performance in pathogen inhibition under laboratory conditions (Neupane et al., 2018). *Trichoderma* fungi can enhance plant resistance against nematode attacks through the activation of hormone-mediated defence mechanisms (Vinale et al., 2008), the synthesis of secondary metabolites, and enzymes and an altered translocation of plant chemical defense components (Poveda et al., 2020). Native *Trichoderma* isolates had the potential ability to reduce the severity of late blight and promote plant growth and tuber yield in potato (Rokaya et al., 2023). A variety of plant metabolites in roots and exuded from roots to the rhizosphere influence nematode behavior, development, reproduction, and even survival (Wang et al., 2018). Some metabolites thus facilitate plant parasitic nematode infection and damage, whereas others directly or indirectly reduce damage. Fosthiazate is an efficient organophosphate chemical that has nematicidal activity against *Meloidogyne* (Li et al., 2020). It impairs nematode synapses ability to function normally, which in turn minimizes the level of root invasion (Huang et al., 2016).The neem cake, a by-product of neem, upon decomposition, produces ammonia, and certain other toxic compounds, which have been reported to exhibit anti-pathogenic properties (Abbasi et al., 2010). These studies support the findings of the research paper provided the information that root-knot nematode can be managed in the protected cultivation (plastic house) with incorporation of *Trichoderma viride*. Neem cake, root growth hormone, well decomposed cow dung slurry and also with the fosthiazate. The *Meloidogyne* species show wide morphological variations among and within species, making their identification difficult. The perineal pattern technique has been used to characterize *Meloidogyne* spp. (Chitwood, 1949), however, it has the problem of only being useful when females are present in the crop, and sometimes more than one species of RKNs are found together in the same plant root or soil.

From the above viewed from different authors during the study suggest the use of different treatments that are applied during the research favors the management of Nematode infestation. Thus, present study mainly focused on the best management option of both chemicals and non-chemical approach in the management option of the pathogen that is supported by the different study conducted on the management of the Root Knot Nematodes in tomato growing crop area.

CONCLUSIONS

The present study concludes that fosthiazate, neem cake, and biogas slurry have been highly effective in comparison with other treatments in reducing root-knot nematode infestation in tomato crop cultivated under protected plastic house conditions. Neem cake and biogas slurry appeared to be good alternatives against Fosthiazate. The additional incentive of soil amendments like neem cake, and biogas slurry along with bio-control agents and root hormones, in addition to its suppressive effect on nematodes, offer a double advantage to the farmers by increasing crop yield. However, the study needs to be conducted in varied conditions with further incorporation of a wide range of treatments.

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