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Impact of various soil amendments on root knot nematodes and plant growth attributes of tomato (*Solanum lycopersicum* L.)

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Abstract

Root-knot nematodes are important plant pests that can cause significant damage to a wide range of crops, leading to yield losses and economic impact. The experiment was conducted to investigate the influence of different organic soil amendments on nematode population and plant growth attributes. The experiment was conducted using various soil amendments, including Farmyard Manure (FYM), Rock Phosphate (RP), Poultry Manure (PM), and Biochar (BC), at two concentration levels. Their effects were assessed based on various plant growth and nematode population parameters. The results indicated the highest gall population was observed in the Farmyard Manure (FYM-5%), with 24.3 galls per plant. Gall index, a measure of nematode infestation severity, showed variation among the treatments. Rock Phosphate (RP-5%) had the highest gall index of 6.4. Poultry Manure (PM-5%) exhibited the highest population, with 39.4 egg masses per plant. The Farmyard Manure (FYM-10%) treatment had the highest nematode population 120 per 250 cm^3 of soil. Female populations in the soil, Poultry Manure (PM-5%) had the highest count, with 65.4 females per 250 cm^3 of soil. The plant growth attributes displayed significant variations depending on the type and concentration of the soil amendment. Plant height was significantly influenced by the type of soil amendment, with the Farmyard Manure (FYM-10%) treatment resulting in the tallest plants (40.5 cm). The number of leaves per plant varied among the treatments, where Poultry Manure (both 5% and 10% concentrations) exhibiting the highest leaves (31 and 36, respectively). Root length (cm) has significant positive correlation with gall index r=0.252 and 0.955 at lower and upper limits respectively. Similarly fresh plant weight (g) had significant positive correlation with egg masses/plant r=0.976 and 0.954 at lower and upper limits respectively.

Keywords: Correlation, Plant growth, Root knot nematodes, Soil amendments, Tomato

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Introduction

Tomato (*Solanum lycopersicum* L.) cultivated globally, is an important vegetable crop and is praised for its health benefits (Shah et al., 2013; Shah et al., 2017; Natalini et al., 2021). It is known to be rich in vitamins (A, B, and C) and provides food with various colors and flavors (Shah et al., 2015; Shah et al., 2016). Recent studies have emphasized their medicinal potential because of the antioxidant properties of ascorbic acid and the quantity of lycopene (Fratianni et al., 2020). Despite its importance, tomato farming encounter challenges, with root-knot nematodes (*Meloidogyne* spp.) representing one of the most deleterious factors (Shilpa et al., 2022). Root-knot nematodes (*Meloidogyne incognita* and *Meloidogyne javanica*) have a broad host range, impacting plant species over 3000 globally and inflicting 5-20% losses in tomato crops in Pakistan (Seid et al., 2015). Tomato is among the agricultural items severely affected by these nematodes, and its infestations have immediate consequences such as reduced uptake of nutrients and water, root tip death from the disease, and blocking of xylem and phloem vessels (Arsad et al., 2020). *M. incognita* infestation has been linked to yield losses ranging from 27-59% (Raza et al., 2016). Root-knot nematodes (RKN) exert a significant impact on plants, manifesting a range of both direct and indirect effects. Directly, RKN interfere with plants by competing for nutrients and water, causing the demise of root tips, and impeding the formation of continuous xylem and phloem cell connections. On the other hand, they indirectly affect plants by establishing physical entry points for pathogens, compromising the host's resistance mechanisms, and heightening susceptibility to foliar diseases (Javed et al., 2007).

The soil is a dynamically significant factor for crop productivity and development (Li et al., 2021). It can be improved in several ways to increase crop yields (Chludil et al., 2008). However, excessive chemical fertilizer use has declined organic matter content and degraded soil structure (Pahalvi et al., 2021). Organic fertilizers play a pivotal role in improving soil quality and development of beneficial soil microorganisms. Numerous research findings suggest a strong correlation between heightened microbial activities in the soil and enhanced agricultural product yields, along with better control over pests and diseases (Zhang et al., 2010). Organic fertilizers are a more environmentally friendly alternative to synthetic fertilizers and increase the fertility of the soil, make the soil structure better, and stimulate the activity of beneficial microbes. Organic fertilizers environmentally safer are generated from sources such as animal waste, compost, and plant residues that have been used in agriculture for a very long time. To address these issues, various soil amendments, including poultry manure, compost, biochar, and crop residues, have been used to improve soil quality and structure and to enhance nutrient availability. These amendments increase the activity of beneficial microbes, provide essential nutrients, and suppress soil-borne pathogens. They increase the soil's water-holding capacity, lower the soil pH, and boost nutrient availability (Kamal et al., 2015).

The utilization of bio-fertilizers and beneficial microbes has a favorable impact on both crop yield and plant growth. The application of organic amendments serves to enhance the organic components in the soil, consequently leading to an augmentation in crop nutrient utilization efficiency and a reduction in the adverse effects of climate change (Liang et al., 2018). Nematode infestations can be economically controlled through the application of organic amendments. Additionally, these amendments contribute to enhancing the overall health of the soil (Briar et al., 2016). Therefore, it was aimed to determine the effects of soil amendments on root-knot nematode infection to evaluate the most effective amendment showing significant results providing cost-effective nematode management methods to farmers.

Materials and Methods

Experiment was carried out at the research area of Department of Plant Pathology, University of Agriculture, Faisalabad, during 2019-20. The aim was to investigate the impact of various novel soil amendments, including Farmyard Manure (FYM) at concentrations of 5% and 10%, Rock Phosphate (RP) at 2.5% and 5%, Poultry Manure (PM) at 5% and 10%, and Biochar (BC) at 0.5% and 1%, on the control of *M. incognita* in Tomato (*Solanum lycopersicum* L.) under Completely Randomized Design (CRD) with three repeats.

Isolation and identification

The plants infested with root knot nematodes were carefully uprooted along with the surrounding soil. Using a trowel, the roots with visible galls were uprooted up to a depth of one foot in polythene bags and shifted to laboratory. The roots were washed gently under clean water to remove any soil particles. The galls containing adult females were placed in a petri dish filled with tap water. Using forceps, the root tissues were carefully exposed adult females using a dissecting needle. The neck of the female was cut using a dissecting needle, and the internal contents were removed to study the perineal patterns of female nematode for identification. Nematode isolation was performed from both the roots and soil using the Baermann funnel method. The roots were cut into small pieces measuring 1 to 2 cm, and half of the pieces were placed in the Baermann funnel. This technique allowed for the isolation of nematode larvae to be examined under microscope to confirm the presence of *Meloidogyne incognita* L. The tomato nursery (cv. Naqeeb) was prepared in seedling trays filled with coconut peat. Proper care and management practices were implemented for the seedlings. After 4-6 weeks, the seedlings were transplanted in the pots.

Inoculation technique

The inoculation process involved preparing three holes in the soil near the roots of the pots, without damaging the roots. These holes were evenly spaced from each other. Subsequently, a suspension containing the 1000 second stage juveniles (J2) was injected into the holes which were covered with soil completing the inoculation procedure.

Data recording

Two months after the inoculation, we carefully extracted the roots of tomato plants from their pots and diligently rinsed them to eliminate any clinging dust particles. To eliminate excess moisture on the root surfaces, a gentle blotting technique with absorbent sheets was used. To measure various parameters related to plant growth, including the number of leaves per plant, plant height (cm), root length (cm), root weight (g), dry root weight (g), as well as the fresh and dry weights of the entire plants. In addition to these plant growth parameters, the chlorophyll content as expressed (mg/g) through the SPAD measurement was measured. Furthermore, the number of galls per plant, the gall index, egg masses per plant, and the nematode population, encompassing both total nematodes and female nematodes per 250 cubic centimeters of soil was assessed. To estimate soil nematode populations, Cobb's sieving method was followed (Ibrahim et al., 2019).

Statistical analysis

The experiment's results underwent statistical analysis using Analysis of Variance (ANOVA) in Statistics software version 8.1. To ascertain significant differences among treatment means, Least Significant Differences (LSD) were calculated at a significance level of P = 0.05. Simple correlations between plant growth parameters and nematode population parameters were calculated.

Results

Different soil modifications, including Farmyard Manure (FYM), Rock Phosphate (RP), Poultry Manure (PM), and Biochar (BC), at two concentration levels on various plant growth and nematode population attributes were investigated during the current study.

Nematodes population variations under various amendments

The nematode population parameters displayed diverse responses to the various soil amendments as shown in Fig. 1. The highest population of root galls/plant was observed in the farmyard manure (FYM-5%) treatment with 24.3 galls per plant, followed by Farmyard manure (FYM-10%) and rock phosphate (RP-2.5%) treatments with 17 and 15.4 galls per plant, respectively. Poultry manure (PM-5%) exhibited the lowest population of root galls/plant with 7.8 galls per plant. Regarding the Gall index, rock phosphate (RP-5%) recorded the highest value of 6.4, while Biochar (BC-0.5%) had the lowest value of 2.0. Among the treatments, poultry manure (PM-5%) showed the highest population of egg masses with 39.4 egg masses per plant, whereas rock phosphate (RP-5%) had the lowest population with 16 egg masses per plant. The nematodes soil population was highest in farmyard manure (FYM-10%) with 120 nematodes, followed by poultry manure (PM-5%) and farmyard manure (FYM-5%) with 95 and 94 nematodes per 250 cm^3 of soil, respectively. In terms of female populations in the soil, poultry manure (PM-5%) exhibited the highest count with 65.4 females per 250 cm^3 of soil, whereas Biochar (BC-0.5%) had the lowest count with 35.4 females.

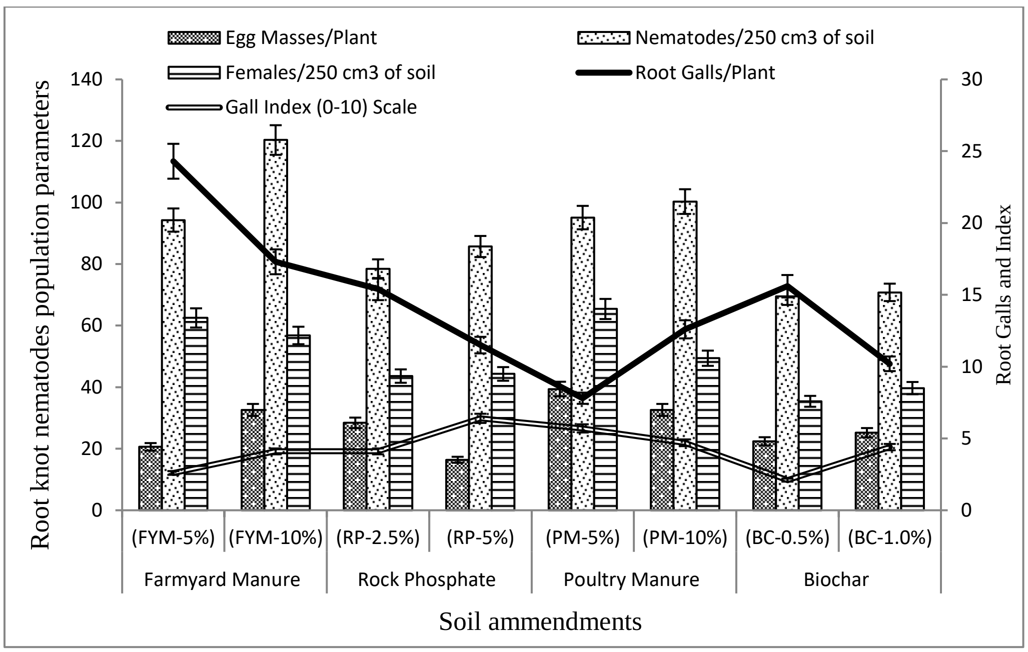

Fig. 1 Responses of nematodes population parameters with different soil amendments

Effects of soil amendments on plant characters

Effects of soil amendments on different plant attributes including plant height, number of leaves, root length, root weight, dry root weight, plant fresh weight, plant dry weight, and chlorophyll levels were also investigated as shown in Fig. 2. The results indicated that the choice of soil amendment significantly influenced plant height. The treatment with "Farmyard Manure (FYM-10%)" resulted in the highest plant height (40.5 cm), followed by "Rock Phosphate (RP-5%)" (39.7 cm), while the "Poultry Manure (PM-10%)" treatment had the lowest plant height (18 cm). Higher concentrations of FYM and RP appeared to positively affect plant height. The impact of farmyard manure (FYM) showed improvement in plant growth criteria. Conversely, the "Farmyard Manure (FYM-5%)" treatment had the lowest number of leaves (23.6 leaves). Regarding root length, the "Rock Phosphate (RP-5%)" treatment displayed the longest roots (21 cm), followed by "Farmyard Manure (FYM-10%)" (15.72 cm) and "Biochar (BC-1.0%)" (17.34 cm). The treatments involving poultry manure and the lower concentration of Farmyard manure showed relatively shorter root lengths. In terms of root weight, the highest values were obtained with the "Farmyard Manure (FYM-10%)" (16 g) and "Rock Phosphate (RP-5%)" (15 g). On the other hand, the "Poultry Manure (PM-5%)" treatment yielded the lowest root weight (9.82 g). Analyzing the results further, the "Biochar (BC-1.0%)" treatment had the highest dry root weight (6 g), while "Rock Phosphate (RP-5%)" and "Farmyard Manure (FYM-10%)" had relatively lower dry root weight values. The "Farmyard Manure (FYM-5%)" and "Poultry Manure (PM-5%)" treatments resulted in the highest plant fresh weight (56 g and 50 g, respectively), while the "Biochar (BC-1.0%)" treatment had the lowest plant fresh weight (32 g). Results regarding the poultry amendment not yielded prominent results in plant growth attributes as compared to the previous findings. Regarding plant dry weight, the "Farmyard Manure (FYM-10%)" treatment had the highest value (54 g), followed by "Farmyard Manure (FYM-5%)" (48.96 g), while the "Rock Phosphate (RP-2.5%)" treatment had the lowest plant dry weight (44 g). The Chlorophyll levels did not follow a clear pattern in relation to the different soil amendments. Lower rates of biochar amendment improved the efficacy against RKN infestation. The choice of soil amendment had a significant impact on various plant growth parameters. The effects varied depending on the type and concentration of the soil amendment. It is important to select appropriate soil amendments to optimize plant growth and productivity. Further research and analysis may be required to gain a more comprehensive understanding of the relationship between soil amendments and plant growth attributes.

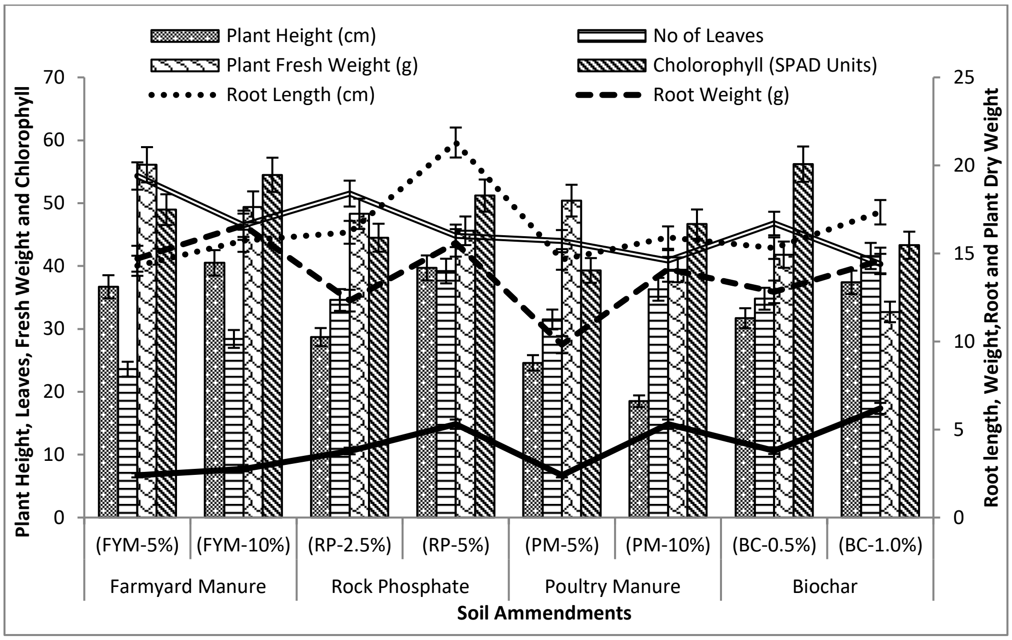

Fig. 2 Responses of plant growth parameters with different soil amendments

Cumulative effect of soil amendments on nematode

The cumulative effect of soil amendments on different root knot nematode parameters and plant growth habits was analyzed. It shows the correlation between different nematode parameters (Root galls/plant, gall index (1-10) scale, egg masses/plant, nematodes/250 cm3 of soil, Females/250 cm3 of soil) and two concentrations of soil amendment treatments: lower limit (ranged 0.5-5%) and upper limit (ranged 1-10%) with plant growth parameters. For the root galls/plant parameter, the lower limit (0.5-5%) of soil amendment treatment had a strong positive correlation (r=0.986) with plant height (cm) and a strong positive correlation (r=0.959) with root weight (g). In contrast, the upper limit (1-10%) had a significant negative correlation (r=-0.916) with the number of leaves/plant. All other parameters did not show a significant correlation with soil amendments as shown in Table 1.

Table 1 Cumulative effect of soil amendments on root galls per plant and plant growth habits (Pearson Correlation + P Value)

Soil Amendments	Plant Height (cm)	No of Leaves/ Plant	Root Length (cm)	Root Weight (g)	Dry Root Weight (g)	Plant Fresh Weight (g)	Plant Dry Weight (g)	Chlorophyll (SPAD Units)
Lower Limit (0.5-5 %)	0.986 (0.014)*	-0.615 (0.385)	-0.367 (0.633)	0.959 (0.041)*	-0.047 (0.953)	0.427 (0.573)	0.888 (0.112)	0.546 (0.454)
Upper Limit (1-10%)	0.315 (0.685)	-0.916 (0.054)*	-0.636 (0.364)	0.781 (0.219)	-0.881 (0.119)	0.557 (0.443)	0.403 (0.597)	0.582 (0.418)

Gall index had a significant positive correlation (r = 0.252) with root length and a significant negative correlation (r = -0.975) with chlorophyll content under lower limits. Under upper limits of soil amendment, a significant positive correlation (r = 0.955) with root length was observed as shown in Table 2.

Table 2 Cumulative effect of soil amendments on gall index and plant growth habits (Pearson Correlation + P Value)

Soil Amendments	Plant Height (cm)	No of Leaves/ Plant	Root Length (cm)	Root Weight (g)	Dry Root Weight (g)	Plant Fresh Weight (g)	Plant Dry Weight (g)	Chlorophyll (SPAD Units)
Lower Limit (0.5-5 %)	-0.798 (0.202)	0.130 (0.870)	0.252 (0.048)*	-0.844 (0.156)	-0.347 (0.653)	0.262 (0.738)	-0.377 (0.623)	-0.975 (0.025)*
Upper Limit (1-10%)	0.370 (0.630)	0.365 (0.635)	0.955 (0.045)*	0.079 (0.921)	0.151 (0.849)	0.337 (0.663)	0.577 (0.423)	0.341 (0.659)

Egg masses/plant had a significant negative correlation (r=-0.923 and -0.978) with plant height and root weight, respectively, under lower limits. A significant positive correlation (r=0.976) with fresh weight/plant was observed under lower limits, while a significant positive correlation (r=0.954) with fresh weight/plant and a significant negative correlation (r=-0.985) with root length were found under upper limits of soil amendment as shown in Table 3.

Table 3 Cumulative effect of soil amendments on egg masses and plant growth habits (Pearson Correlation + P Value)

Soil Amendments	Plant Height (cm)	No of Leaves/ Plant	Root Length (cm)	Root Weight (g)	Dry Root Weight (g)	Plant Fresh Weight (g)	Plant Dry Weight (g)	Chlorophyll (SPAD Units)
Lower Limit (0.5-5 %)	-0.923 (0.057)*	0.262 (0.738)	0.180 (0.820)	-0.978 (0.022)*	-0.316 (0.684)	0.976 (0.024)*	-0.680 (0.320)	-0.852 (0.148)
Upper Limit (1-10%)	-0.485 (0.515)	-0.664 (0.336)	-0.985 (0.015)*	0.031 (0.969)	-0.465 (0.535)	0.954 (0.046)*	-0.329 (0.671)	0.981 (0.019)

Nematodes/250 cm^3 soil had a significant negative correlation (r=-0.959) with dry root weight under lower limits, and a significant negative correlation (r=-0.971 and -0.940) with the number of leaves/plant and dry root weight, respectively, under upper limits of soil amendment as shown in Table 4. Females/250 cm^3 soil had a significant negative correlation (r=-0.973) with dry root weight and a significant positive correlation (r=0.975) with dry weight of the plant under lower limits. Under upper limits of soil amendment, a significant negative correlation (r=-0.974 and -0.944) with the number of leaves/plant and dry root weight, respectively, was observed as shown in Table 5. The statistical results in Fig. 3 having LSD (0.05) value and CV (%) revealed significant differences among the treatments for the measured parameters. Differences exceeding the LSD values (2.35, 0.36, 1.89, 7.51, and 4.82) are considered statistically significant. These differences can be attributed to the varying effects of the soil amendments on plant growth and development. Therefore, the choice of soil amendments and their concentrations had a significant impact on the parameters related to root health, plant growth, and chlorophyll levels.

Table 4 Cumulative effect of soil amendments on nematodes/250 cm3 of soil and plant growth habits (Pearson Correlation + P Value)

Soil Amendments	Plant Height (cm)	No of Leaves/ Plant	Root Length (cm)	Root Weight (g)	Dry Root Weight (g)	Plant Fresh Weight (g)	Plant Dry Weight (g)	Chlorophyll (SPAD Units)
Lower Limit (0.5-5 %)	-0.031 (0.969)	-0.767 (0.233)	-0.622 (0.378)	-0.272 (0.728)	-0.959 (0.041)*	0.880 (0.101)	0.120 (0.899)	-0.672 (0.328)
Upper Limit (1-10%)	-0.074 (0.926)	-0.971 (0.029)*	-0.532 (0.468)	0.548 (0.452)	-0.940 (0.030)*	0.792 (0.208)	0.473 (0.527)	0.770 (0.230)

Table 5 Cumulative effect of soil amendments on females/250 cm3 of soil and plant growth habits (Pearson Correlation + P Value)

Soil Amendments	Plant Height (cm)	No of Leaves/ Plant	Root Length (cm)	Root Weight (g)	Dry Root Weight (g)	Plant Fresh Weight (g)	Plant Dry Weight (g)	Chlorophyll (SPAD Units)
Lower Limit (0.5-5 %)	-0.069 (0.931)	-0.748 (0.252)	-0.649 (0.351)	-0.318 (0.682)	-0.973 (0.027)*	0.841 (0.159)	0.975 (0.025)*	-0.664 (0.336)
Upper Limit (1-10%)	-0.063 (0.937)	-0.974 (0.026)*	-0.533 (0.467)	0.557 (0.443)	-0.944 (0.056)*	0.793 (0.207)	0.477 (0.523)	0.772 (0.228)

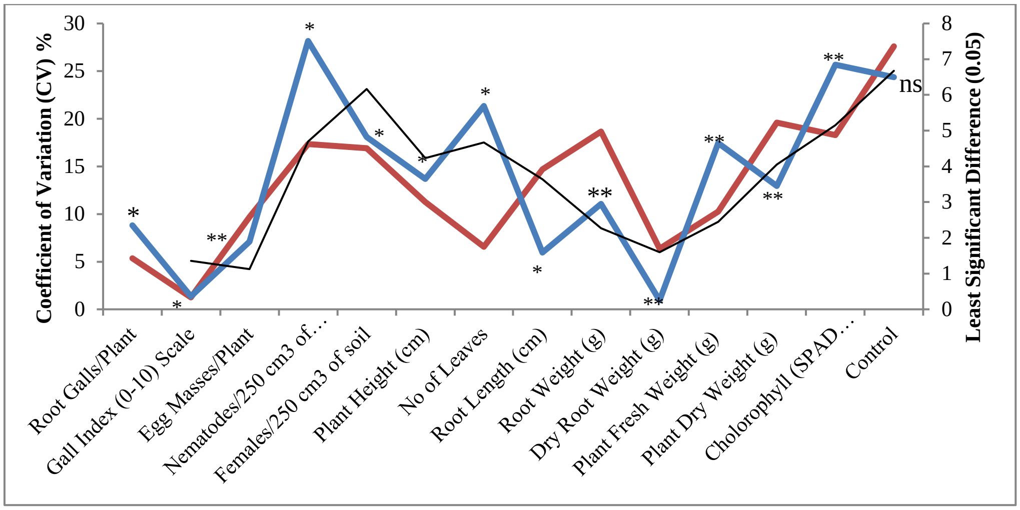

Fig. 3 Mean values and CV (%) values indicting responses of all parameters

Discussion

Several studies regarding the relation of soil amendment and root knot nematode in the past have confirmed our findings. The effectiveness of various biological wastes i.e., sawdust, refuse dump and rice husk in managing root knot nematodes revealed that the refuse dump treatment exhibited the highest significant reduction in nematode population (Hassan et al., 2010). The incorporation of poultry litter did not have a significant impact on the soil population levels of *M. incognita* which differs our findings However, the inclusion of broccoli resulted in a 36% reduction in root galling (Lopez-Perez et al., 2010). Chicken manure has the potential to enhance the effectiveness of bio fumigation in controlling *M. incognita* infestation (Lopez-Perez et al., 2005). Biochar at higher limit had significant impact on nematode population variation which was not supported by Mondal et al. (2021) who revealed that neither biochar nor vermicompost exudate exhibited direct killing effects on the nematode. Some other findings gave support to the current results where the application of biochar resulted in a decrease in root galling and a significant improvement in the growth and yield of tomato plants. As the concentration of biochar increased, there was decrease in the formation of nematode galls. (Ibrahim et al., 2019).

Biochars had the potential to enhance resistance against *M. incognita*. 3% biochar resulted in significant improvements in plant morphological traits, including shoot length, shoot fresh weight, and dry weight. This concentration of biochar exhibited the highest reduction in the number of galls, egg masses, and females associated with *M. incognita* infestation (Arshad et al., 2020). These findings underscore the varying effects of different soil amendments on the nematode population parameters, emphasizing the importance of selecting appropriate amendments based on the desired outcomes. The average plant height, total plant fresh weight, shoot dry weight, leaves dry matter, and total yield of tomato fruit were 89.92%, 68.9%, 75.34%, 59.4%, and 71.1% respectively (El-Gazar et al., 2013). The application of biofertilizers yielded maximum plant height of 136.24 cm, the highest number of fruits per plant (31.13), fruit weight per plant (1.35 kg), number of seeds per fruit (81.93), seed yield per plant (3.04 g), and seed yield per plot (36.58 g) (Angadi et al., 2017) which is in accordance with our findings. The impact of potassium humate (KH) combined with farmyard manure (FYM) on the growth, fruit yield, and quality of tomato plants showed significant improvements in shoot dry weight, root dry weight, leaf mineral composition, total fruit yield, fruit vitamin C and total soluble solids (TSS) contents (Mostafa, 2011).

The effects of different levels of potassium fertilizer in combination with Farmyard Manure (FYM) on tomato plants showed highest tomato yield of 39.05 t/ha was obtained in the pots treated with FYM. Furthermore, the use of FYM and potassium fertilizer had notable effects on key growth parameters, including the number of flowers per plant, number of fruits per plant, and fruit diameter (Khan et al., 2013). In terms of the number of leaves, the treatments involving poultry manure (both 5% and 10% concentrations) showed the highest leaf numbers (31 and 36 leaves, respectively). Rock Phosphate (RP-2.5%) and Rock Phosphate (RP-5%) also exhibited relatively higher leaf numbers (34.6 and 39.2 leaves, respectively). The effects of different phosphorus enrichment sources, specifically rock phosphate on tomato seedlings indicated its capability to serve as an effective phosphorus source for promoting the growth of tomato seedlings. (Mihreteab et al., 2016). The application of poultry litter had a significant impact on increasing soil organic matter content. Additionally, the plants treated with PL demonstrated higher fresh and dry weights (Akca & Namli, 2015). Additionally, the application of biochar at these lower rates promoted the overall performance of tomato plants. (Agyemang, 2022).

Effects of poultry manure, cow dung, and the nematicide carbofuran were compared. The results indicated that the highest application rates significantly ($P \leq 0.05$) reduced the root gall-index and nematode population density in the soil (Amulu & Adekunle, 2015). The impact of poultry manure and rapeseed cake on root-knot nematode infestation and tomato yield demonstrated a significant interaction influence on number of eggs/egg masses, root galling, population of J2 nematodes, as well as the yield and growth of the tomato crop (Shiferaw et al., 2017). Where poultry manure resulted in the highest vegetative growth (119.07 g/plant) and tomato yield (133.42 g/plant). Even in the presence of nematode challenge these findings highlight the resilience and effectiveness of poultry manure in promoting plant growth and productivity (Abolusoro et al., 2020). Biochar amendment at a 20% application rate revealed a positive impact on the morphology of plant roots, shoots, and leaves, especially during the vegetative and fruit stages of growth (Simiele et al., 2022). All other parameters did not show a significant correlation with soil amendments. Mono super phosphate (MSP) had a significant positive impact on all growth parameters of tomato shoots and roots, as well as on yield quantity and quality. While the use of Rock phosphate (RP) resulted in the lowest values for tomato growth and yield (Gad & Kandil, 2010). The observed variations in these parameters highlight the importance of carefully selecting and optimizing the use of soil amendments to promote desirable plant growth and effectively manage root health.

Conclusion

This study highlights the varying responses of nematode population parameters to different soil amendments, underscoring the importance of selecting suitable amendments for achieving specific outcomes. Among the tested soil amendments, farmyard manure (FYM) at a concentration of 10% demonstrated the highest efficacy in enhancing the nematode population showed strong positive correlation between the populations of root galls per plant and plant height. These findings suggest that taller plants were more favorable for nematode population development. It's important to mention that things like how plants grow their roots and the number of leaves they have can indirectly affect nematode populations. This is because they provide more resources and places for nematodes to live

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