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Original Research Article

INNOVATIVE UTILIZATION OF SOIL AMENDMENTS FOR IMPROVING RESISTANCE IN OKRA AGAINST MELOIDOGYNE INCOGNITA

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Abstract: *Okra productivity is significantly hindered by various pests and pathogens, including bacteria, fungi, and nematodes, particularly Meloidogyne incognita, which poses a severe threat to plant growth and yield. This study aimed to evaluate the efficacy of different organic and inorganic soil amendments—poultry manure (PM), farmyard manure (FYM), biochar, and rock phosphate (RP)—in managing Meloidogyne incognita in okra cultivation. A completely randomised design (CRD) with factorial arrangements and three replications was used to test two concentrations of each amendment: FYM (5%, 10%), biochar (0.5%, 1%), RP (2.5%, 5%), and PM (5%, 10%). Soil treatments were analysed for their effects on key growth parameters, including root weight, shoot weight, shoot dry weight, root length, plant height, number of leaves, and chlorophyll content. The findings revealed that FYM at 5% significantly enhanced root weight, shoot weight, and shoot dry weight ($p \leq 0.05$). PM at 5% showed the greatest impact on root length, while FYM at 10% resulted in the highest plant height. Additionally, FYM at 5% contributed to the maximum number of leaves and chlorophyll content. Farmyard manure demonstrated superior performance compared to poultry manure, biochar, and rock phosphate in improving growth parameters and mitigating the effects of Meloidogyne incognita in okra cultivation. FYM at 5% and 10% concentrations proved to be the most effective treatments, suggesting its potential as a sustainable management strategy for nematode infestations in okra.*

Keywords: Root-Knot Nematode, Abelmoschus Esculentus, Confrontation, Farmyard Manure, Poultry Manure, Rock Phosphate,

Introduction

Okra (*Abelmoschus esculentus L.*) is a cost-effective, allopolyploid vegetable crop that is cultivated worldwide. Okra is cultivated for its fresh flowers, fruits, seeds, buds, roots, and pods used for food, feed, shelter and fire for cooking meals in the geographical regions (Mihretu *et al.*, 2014). Okra fruit contains minerals such as iron, magnesium, potassium, and calcium and is enriched with vitamin A and C, protein, antioxidants and polysaccharides. Okra seeds are antispasmodic and roots contain a high mucilage level which is used for plasma replacement. Seeds of okra comprised of high oil content (20-40%) compared to other oilseed crops and it is potentially grown as oil seed crop (Santos *et al*. 2013). Plant-borne nematodes constitute a serious threat to the global cultivation of okra. Among the entire destructive pathogens, *Meloidogyne incognita* is wildly devastating and substantial nematode specie infecting okra crop. There have been estimates varying from US$ 80-150 billion in yield reductions of Plant-parasitic nematodes (PPNs) annually (Nicol *et al.*, 2011; Ali *et al.*, 2017). At Pakistan, *Meloidogyne incognita* is the dominant specie found in all growing regions of okra, affecting yield and productivity (Mukhtar *et al*. 2013). The PPNs cause hyper proliferation leading to cell cycle modification and

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escalate the parasitised cell's size. This approach fosters integrated proximity and reduced the plant vegetative growth and harvest (Carneiro *et al*., 2002; Lu,2014) due to the formation of nematode alimentary sites (gall cells) (Shukla *et al*., 2018, Antonino de Souza Junior, J.D. *et al* .2017) disrupts the uptake of nutrients and water. Root tangle nematodes (Meloidogyne spp.) are polyphagous, sedentary endo-parasites which cause damage to economically important crops globally (Trudgill *et al*., 2001; Abad *et al*., 2008). Life cycle of *M. incognita* involves 6 stages viz. egg, J1 (1st-stage juvenile), J2 (2nd-stage juvenile), J3 (3rd-stage juvenile), J4 (4th-stage juvenile), and adults (female and male). Eggs and J2 are exophytes whereas J3, J4 and female are usually sedentary endoparasite (Abad *et al*., 2008).

To-date, there are a few of economically feasible viable management strategies i.e. chemical control, cultural and resistant cultivar has restricted the management efficacy of nematodes. Biological/organic soil amendments could be a potential option instead of chemical to control plant pathogens. Soil amendments are one of the best biocontrol measures to restrict the plant-parasitic activity of nematodes in spite of chemicals that are hazardous to crop as well as to environment (Akhtar and Malik, 2000). To control hyto parasitic nematodes, soil amendments have a positive effect on the biological, chemical, and physical properties of soil and enhance the yield (Ntalli et al., 2020). The organic amendment is polysemic as it includes a variety of products containing green manures from crop residues or cover crops, animal dung (poultry, cattle), town wastes or industrial wastes (oilseed cakes). Furthermore, by releasing specialized nematocidal chemicals, soil nutrient uptake and water holding capacity are improved, ultimately leading to improved plant vigor and better tolerance against nematodes. Escalation of microbial activity antagonist to nematode responds directly by organic amendments and indirectly stimulates the parasites, predators (e.g. nematophagous fungi, micro-arthropods, parasitic bacteria) of nematode. Currently, scientists are focusing on developing integrated strategies to combat the pest because, frequently, a single strategy may not be sufficient to control plant nematodes. This includes managing the nematodes through the use of organic amendments (Faruk, 2019, Gomes et al., 2018). Several researchers investigated the application of organic's amendments to suppress parasitic infection (Sidhu and Kanwar, 2020; Ntalli et al., 2020). Organic amendments including chicken, cattle, horse and sheep manure were effective against plant-parasitic nematodes in various crops (Ismail and Mohamed, 2012; Renco, 2013; Abdel-Bary *et al.*, 2014). The organic amendments have beneficial effects on soil physical conditions, nutrients, crop viability and biological activity. The use of organic manures results in improved crop production and quality (Sidhu and Kanwar, 2020). Even though organic manure contains growth-promoting components such as hormones and enzymes, as well as plant nutrients, makes it important for soil fertility and productivity. Amendments such as farmyard, green manure, and poultry manure can be mixed into the soil to check the response of nematodes (Collange *et al*. 2011).

Considering the significance of organic substances, the current investigation was conducted to examine the influence of organic soil amendments on plant growth and production parameters under *Meloidogyne incognita* infection.

Methodology

Experimental Layout

The experiment was performed in the research area of Plant Pathology, University of Agriculture Faisalabad. The seeds of a variety Sabazpari were collected from Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan. In order to meet the present study objectives, varying levels of farmyard manure, biochar, rock phosphate and poultry manure were used. Rock Phosphate and biochar were obtained from Soil Microbiology and Biochemistry Laboratory, Institute of Soil and Environmental Sciences (ISES), University of Agriculture Faisalabad (UAF). Poultry manure and farmyard manure were collected from the Institute of Animal Husbandry, University of Agriculture Faisalabad. In Table 1, treatments plan of present study with completely randomized design (CRD) - factorial arrangements were presented.

Table 1: Treatments Plan Used for Research Work

Treatments	level – I	level –II
Farmyard manure	5%	10%
Rock phosphate	2.5%	5%
Biochar	0.5%	1%
Poultry manure	5%	10%
Negative	1	1
Positive	1	2

Positive Control = Nematode applied
Negative Control= Nothing applied

Raising okra nursery

Seeds were sown in the seedling trays containing peat moss and trays were placed in a greenhouse. Before being used in the experiment, the seedlings were cultivated in these trays for 3 weeks. Peat moss provides nutrient holding capacity and better aeration to the soil. The precautionary measures were adopted for seedling care in which pruning of weeds were involved. Irrigation was done 2 times a day.

Preparation of soil and filling of pots

Initially, the soil was spiked with 2 levels of each used amendments including FYM was 5% and 10%, biochar was 0.5% and 1%, rock phosphate 2.5% and 5%, and poultry manure was 5% and 10%.Then the amended soil was shifted to 100 pots in such a way that 20 pots were filled with each treatment viz 2 replications. The same numbers of pots were separated for positive and negative control in which soil was not amended. In positive control, pots were inoculated with inoculum of nematode and whereas only water is applied in the negative control.

Transplantation

After 6 weeks seedlings were transplanted to the pots in the research area of plant pathology. Transplanting was done after 6 weeks in pots containing amended soil with different concentrations. The seedlings were uprooted manually and then embedded the roots in pots containing amended soil.

Assessment of physiological and yield parameters

The numbers of leaves were counted manually and the data was recorded weekly. The progress was observed between each weekly data and the difference was compared. The

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chlorophyll content was measured on weekly basis from leaves with the help of chlorophyll meter (Wellburn, 1994). Plant height of each treatment was measured by measuring scale in cm from the base to the tip of the plant subsequently every week. The okra fruit was collected from each plant and the data was recorded by taking out the average of fruits per treatment for number of fruits per plant. After the counting of fruits, the weight of fruits per plant was taken through weighing balance and the data was recorded in grams and observe the weekly progress. After taking the data of the above parameters, uprooting was done. Root length was measured with a measuring scale in centimeters and the data was collected and compare the difference. After taking the root length, the fresh weight of shoot and root was taken by weighing balance which was recorded in grams. For drying purposes, the roots were cut and wrapped in an envelope paper and then put in dehydrator or oven at 60-70ºC for 48 hours. After overnight, weighing of all dry roots and shoots was done by using a weighing balance and then data was recorded in grams.

Nematodes Characters

Number of females and egg masses

Infected roots were collected for counting the number of females and egg masses. Instead of washing, the roots were dipped in water to lower the risk of losing egg masses. To count the female, the staining method was used in which roots were dipped for 15 to 20 minutes in staining solution of phloxin B (15 mg L^{-1}) which facilitates the counting of egg masses (Hartman and Sasser, 1985). The gelatinous matrix was used for staining and the excess stain was removed by dipping the roots in 1L water. The portion which was stained pink is the indication of female presence. An X12.5 dissecting microscope was used for counting of female and egg masses, and data was recorded.

Assessment of galling and egg masses index

It is a simple technique to assess the roots galling and egg masses index. The damage was observed by the extent of galls on the root system which affect the plant vigor. When the infestation is heavy, it is hard to count the mature galls or egg masses. In this situation, the rating scale (0-5) of Taylor and Sasser was adopted through which root galling and egg masses were easily assessed. The rating scale of galls is shown in Table 2:

Table 2: Rating Scale to Estimate Galls

Score / Rating	Description/Details
0	No galls
1	1-2 galls
2	3-10 galls
3	11-20 galls
4	30 galls
5	Above 30

Statistical Analysis

For the analysis of variance (ANOVA), the complete randomized design (CRD) and factorial design were applied. The means were compared using least significant difference (LSD) test with a 95% level of confidence by using Statistics v. 8.1 (Steel et al., 1997).

Results

In present study, ANOVA (Table 3) indicated that root length (RL), root dry weight (RDW) and fruit weight (FW), while shoot fresh weight (SFW) and shoot dry weight (SDW) showed non-significant response towards treatments and concentration including root dry weight (RDW) treatments. Moreover, a significant effect of treatments in observed in the case of root length (RL), root fresh weight (RFW) and fruit weight (FW). Interaction of treatments with concentration revealed highly significant effect on root fresh weight (RFW), shoot fresh weight (SFW) and shoot dry weight (SDW), whereas concentration only showed highly significant response in root fresh weight (RFW).

Treatments showed highly significant effect while interaction of treatments and concentration respond non-significantly towards nematodes parameters including no. of females, egg masses (EM), egg masses index (EMI), galling (G) and galls index (GI). Non-significant results are also observed in concentration except GI and EMI which represent significant results shown in Table 4.

The observed data of treatments and concentration regarding the effect of different soil amendments applied at variable concentration on plant growth parameters against nematode are given in Table 5. For the treatments and concentrations maximum fresh and dry root weights were observed in positive control with statistically no significant effect of treatments. Furthermore, poultry manure showed significant effect on fresh and dry shoot weights at increasing concentrations with maximum percentage 26.2%, 6.8%, 16.4% respectively) among all tested treatments. Considering the fruit weight, biochar increased the fruit weight percentage by 24.7% followed by rock phosphate (24.7%). Moreover, 2nd concentration proved more effective for fresh and dry root and shoot weights, whereas root length and fruit weight was enhanced pointedly ($p \leq 0.05$) by 1st concentration.

Interaction of treatments and concentration for farmyard manure (10%) elucidate maximum root fresh weight, shoot dry weight and fruit weight with increasing value of 10.6%, 8.8% and 19.0%. Poultry manure 10% significantly maximized the dry root weight. Rock phosphate 2.5% showed the highest fresh shoot weight (30.5%) and root length (14.4%).

Response of various soil applied amendments against nematodes establishment and reproduction attributes (no. of females, no. of galls, galling index, egg masses, egg masses index) are presented in Table 6. Results indicated that nematode attributes were significantly ($p \leq 0.05$) different in all applied soil amendments. Among all the treatments, farmyard manure considerably reduced the no. of females, no. of galls, galling index, egg masses and egg masses index by mean value of 18.5, 8.6, 2.1, 24, 4.7, respectively. Maximum no. of females and galls were examined in rock phosphate which were 21.3, 10.3. The highest mean value of egg masses (29.2) and galling index (2.6) were recorded for poultry manure and biochar. Poultry manure and biochar share the similar the highest mean value for egg mass index (5.2). Furthermore, positive control displayed the maximum mean values for nematode parameters whereas negative control showed no infection due to absence of nematode inoculum. Moreover, 2nd concentration of all the incorporated soil amendments showed the highest mean value of 26.3, 12.4, 2.3, 31.4, 4.7, respectively; which proved the most effective to overcome nematode population. Interaction of treatments and concentrations do not showed significant differences among mean values of nematode parameters. Minimum no. of females, galls and galling index was recorded in farmyard manure 5% with mean value of 17.8, 8.4, 2, respectively, while farmyard manure 10% represent the lowest egg masses and egg masses index. Furthermore, positive control displayed the maximum mean values for nematode parameters whereas negative control showed no infection due to absence of nematode inoculum.

Table 3: Anova for Assessing the Impacts of Soil Amendments in Okra to Improve Resistance against Meloidogyne Incognita

SOV	DF	RFW	RDW	SFW	SDW	RL
Treatment	5	8.386*	0.2764ns	74.24ns	4.69ns	45.026*
Concentration	1	31.248**	0.309ns	53.39ns	2.08ns	4.266ns
Treatment × Concentration	5	16.86**	0.0725ns	213.63**	13.93**	6.346ns
SOV	DF	No. of Females	No. of Galls	GI	EM	EMI
Treatment	5	6643.59**	1274.79**	15.2167**	61813**	62.2667**
Concentration	1	5.4ns	13.07ns	1.35*	12.15ns	0.6
Treatment × Concentration	5	1.08ns	4.31ns	0.39ns	9.67ns	0.32ns

***= Highly significant, * = Significant, ns = Non-significant*

Table 4: Effect of Applied Amendments on Growth and Yield Components of Okra Plants to Enhance Resistance against Meloidogyne incognita

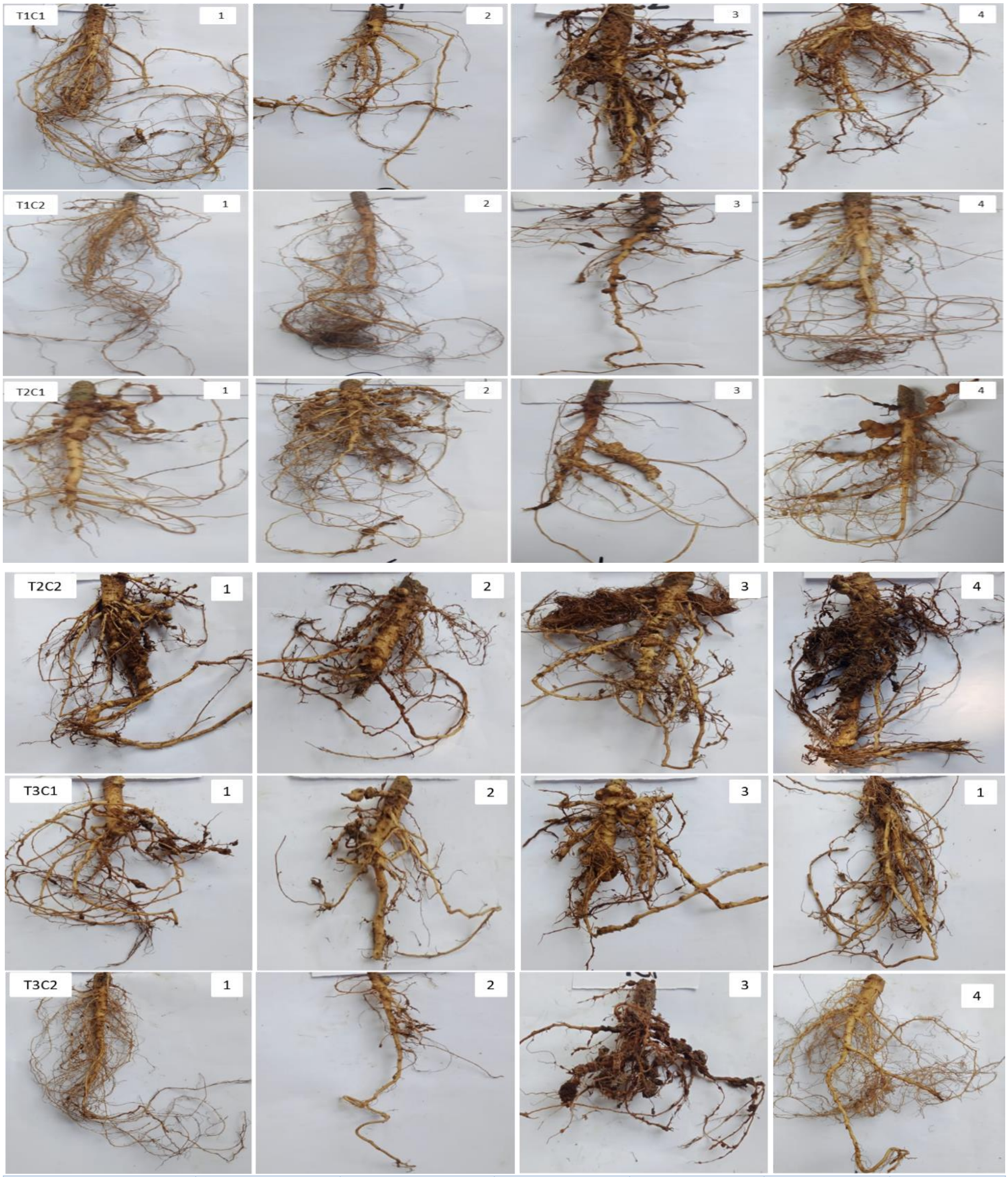
Amendment	RFW	RDW	SFW	SDW	RL	FW
FYM	7.7 ab	1.20 ab	26.1 a	6.5 ab	12.0 bc	20.7 b
PM	7.0 b	1.34 a	26.2 a	6.8 a	16.4 a	17.1 bc
RP	7.2 ab	1.35 a	25.4 a	6.3 ab	13.8 ab	20.6 b
Biochar	5.7 c	1.29 ab	22.2 ab	5.5 bc	11.6 bc	24.7 a
Negative	6.6 bc	0.95 b	22.2 ab	6.7 ab	10.3 c	18.1 bc
Positive	8.4 a	1.42 a	19.5 b	5.1 c	12.3 bc	15.8 c
Concentration 1	6.4 b	1.18 a	22.7 a	6.0 i	13.0 a	21.0 a
Concentration 2	7.8 a	1.33 a	24.5 a	6.3 i	12.4 a	18.0 b

Table 5: Interactive Effects of Applied Treatment and their Concentrations on Okra Plants to Enhance Resistance against Meloidogyne incognita

Treatments	Level	RFW	RDW	SFW	SDW	RL	FW
FYM	5%	4.9 e	1.08 ab	16.9 e	4.23 e	13.6 abcd	22.3 b
FYM	10%	10.6a	1.32 ab	35.4 a	8.85 a	10.4 d	19.0 bc
PM	5%	5.7 de	1.23 ab	25.5 bcd	6.89 bc	15.6 ab	19.7 bc
PM	10%	8.3 b	1.45 ab	26.8 bc	6.71 bcd	17.2 a	14.6 c
RP	2.5%	6.3 de	1.15 ab	30.5 ab	7.72 ab	14.4 abc	22.3 b
RP	5%	8.1 bc	1.55 a	20.3 de	5.06 de	13.2 bcd	18.9 bc
Biochar	0.5%	6.5 cde	1.29 ab	21.4 cde	5.36 cde	11.8 bcd	27.9 a
Biochar	1%	4.9 de	1.29 ab	23.1 cd	5.81 cde	11.4 cd	21.6 b
Negative	1	6.6 cd	0.95 b	22.2 cde	6.70 bcd	10.3 d	18.1 bc
Negative	2	6.6 cd	0.95 b	22.2 cde	6.70 bcd	10.3 d	18.1 bc
Positive	1	8.4 b	1.42 a	19.5 de	5.10 de	12.3 bcd	15.8 c
Positive	2	8.4 b	1.42 a	19.5 de	5.10 de	12.3 bcd	15.8 c

Table 6: Interactive Effects of Amendments and their Varying Levels on Nematode Parameters

Amendments	Concentration	No. of females	No. of galls	GI	EM	EMI
FYM	5%	17.8 b	8.4 c	2.0 d	24.8 bc	4.8 cd
FYM	10%	19.2 b	8.8 c	2.2 cd	23.2 c	4.6 d
PM	5%	19.8 b	8.4 c	2.2 cd	28.6 bc	5.0 bcd
PM	10%	21.2 b	9.8 bc	2.2 cd	29.8 b	5.4 bc
RP	2.5%	21 b	8.6 c	2.0 d	28.2 bc	5 bcd
RP	5%	21.6 b	12 b	2.8 bc	29.8 b	5.2 bcd
Biochar	0.5%	19.8 b	9.8 bc	2.2 cd	25.4 bc	4.8 cd
Biochar	1%	20 b	10.2 bc	3.0 b	29.6 b	5.6 b
Negative	1	0.0	0.0	0.0	0.0	0.0
Negative	2	0.0	0.0	0.0	0.0	0.0
Positive	1	76 a	33.6 a	3.8 a	76.4 a	7.6 a



Positive	2	76 a	33.6 a	3.8 a	76.4 a	7.6 a

Figure 1: Pictorial presentation of nematode galling in four treatments with positive and negative control in response to *M. incognita* infection in okra

Discussion

Among all destructive pathogens, *Meloidogyne incognita* is the most dangerous nematode specie that infects the crop plants (Kayani *et al.*, 2012a, 2012b, 2013; Hussain *et al.*, 2012). Due to Root-knot nematodes, annual crop losses are around100 $ billion globally (Brand *et al.*, 2010). Hence, effective, environmentally friendly and affordable alternatives are required instantly for the immediate control of plant parasitic nematodes. Several nematode control strategies have been implicated successfully comprising nematicides, crop rotation, resistant varieties, biological

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control (parasites, predators) and non-host crops but each method has certain limitations (Akhtar and Malik, 2000). Therefore, soil amendments are a significant tool to control nematodes. Root-knot nematodes cause significant damage to okra, and most cultivars are sensitive to *M. incognita*. In past, several scientists investigated how different okra cultivars reacted to root-knot nematodes. The use of innovative soil amendments to improve resistance to *Meloidogyne incognita* can be an effective control strategy, and the current study conducted at a wide spectrum of organic matter (Tables 4, 5 & 6).

Organic matter reduces the prevalence of the diseases resulting from variety of pathogens of pathogens notably phyto-parasitic nematode, soil borne pathogens and bacteria besides positive effect on yield and growth of plant. In consonance with present study, significant effects of organic amendments against *Meloidogyne spp*. have been reported in the literature for instance D'Addabbo et al. (2000) observed the reduction of M. incognita population by the incorporation of chicken manure on tomato (Sharma *et al., 1997*) by employing mustard straw, hyacinth compost, asparagus compost and ricestraw; grape pomace (Abdel-Bary *et al.,* 2014); via olive pomace (D'Addabbo et al., 2011) or pepper crop residues (Buena et al., 2007). Applications of organic amendments to the soil have positive agronomical effect on chemical, biological and physical properties of soil and on plant growth (Davey *et al.,* 1996). Likewise, many organic materials are chief source of elements which are essential for the plant growth such as copper, magnesium, zinc as well as provide nutrients including nitrogen, calcium, and phosphorus.

Chemicals and nematicides are expensive, limited and have a hazardous effect on the environment and human health also. It is suggested from the present study that the use of organic amendments can lead to the effective control of root-knot nematode (Fig. 1) as it is an environmentally friendly alternative to nematicides and chemicals. As it is a traditional agricultural practice that improves the chemical and physical properties, soil structure, humidity, temperature and nutrient contents which are essential for plant growth. Linford *et al.* (1938) proposed that the soil amendments stimulate the behavior of naturally occurring nematode pest antagonists which control the plant-parasitic nematodes. Organic amendments such as farm manure, green manure and poultry manure are mixed into the soil to increase the potential of soil (Collange *et al*., 2011).

In the current study, we have used different organic amendments to control the root knot nematodes in okra crop. Mean comparison and ANOVA was done to evaluate the effect of treatment, concentration and their interaction for growth, morpho-physiological, and yield attributes against root-knot nematode (Table 3). The findings indicated that there was a substantial effect ($p < 0.05$, Table 3) of 5% concentration of farmyard manure on root weight, shoot fresh and dry weight. Multiple nutritional shortages can be remedied by using farmyard manure. The FYM considerably affect the cation exchange capacity of soil, and the higher rates of applied FYM have also increased soil organic carbon and potentially mineralizable nitrogen. In addition, soil surface (0-15 cm) pH significantly ($p \leq 0.05$, Table 3) increased with manure.

Likewise, root length and nematode characteristics including gall count, female count, and egg mass were most affected by poultry dung at a concentration of 5% (Table 6). Improvement in the fertility of soil and release of nematode toxic substances on decomposition is associated with organic amendments which increases yield (Riegel and Noe, 2000; Oka, 2010). *M. incognita* juveniles have been killed by these toxins in the amended soil. Phenolic compounds and nitrogen have been revealed to be in high concentration in organic manure (Agyarko *et al*., 2006; Renco and Kovacik, 2012). Ammonia is the final product upon the decomposition of nitrogen (Lazarovits *et al.,* 2001; Adetiloye et al., 2006; Oka, 2010; Thoden *et al*., 2011) which kills numerous nematode spp. in the soil have been reported (Lazarovits *et al*., 2001). Moreover, plant parasitic nematodes are additionally reported to be fatally affected by phenolic compounds (Nwaguma and Fawole, 2004).

The negative control showed no damage in which inoculum was not applied. From the mean comparison weekly data 5% of the farmyard showcased the maximum leaves count and chlorophyll content, while 10% of the farmyard exhibited the maximum heights of plants (Tables 4 & 5). These outcomes could be the result of decaying matter, nitrogen availability, and an appropriate C: N ratio for the plant. Moreover, major nutrients including Ca, K, and P, N, S and micronutrients which are important for plant growth are supplied by farm yard manure. The present findings are consistent with those attained by Huosman *et al.* (2016) on garlic and on okra (Subrahmanvam *et al.,* 2011; Kibria et al., 2013). The overall findings indicate that farmyard manure (FM) outperformed than other treatments i.e., biochar (BC), rock phosphate, and poultry manure (PM) against *Meloidogyne incognita* in okra.

Conclusion

Soil amendments recover soil fertility and plant development by stimulating the growth of soil microbiota and decreasing the population of Plant-borne pathogens or worm-like nematodes. In present study, the most of findings have concentrated on different forms of organic amendments as inhibitors of economically important plant-borne parasitic nematodes, especially root-tangle nematodes. The current study's findings indicated that fresh root weight as well as fresh and dry shoot weights were substantially affected by farmyard manure at a concentration of 5%. Similarly, poultry manure with concentration 5% showed a maximum effect on root length. The nematode characters such as number of galls, number of females and egg masses were found maximum in positive control in which inoculum was applied. The negative control showed no damage in which inoculum were not applied. Thus, it is suggested that farmyard manure performed better than other tested amendments (i.e., BC, PM and rock phosphate) against *Meloidogyne incognita* in okra.

Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department Concerned.

Consent for publication
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Conflict of interest

The authors declared absence of conflict of interest.

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