Original Research Article

GROWTH ENHANCEMENT OF OKRA INFESTED WITH *M. incognita*, THROUGH SOIL AMENDMENT WITH NEEM CAKE, POULTRY MANURE AND *Trichoderma Haziarnum*

Abstract

Aims: To evaluate the efficacy of *Trichoderma harzianum,* Neem cake,and poultry manureon the growth performance of okra infected with *M. incognita*.
Study design: The experiment was laid out in a completely randomized design.
Place and Duration of Study: Department of Botany, University of Calabar, between April 2022 and August, 2022.
Methodology: Soil samples were collected at the depth of 0-15cm using auger, heat sterilized and spread out in a shade for 7days to cool before use for planting okra seed. Galled roots of okra plants were blended and emptied into 1000ml beaker. This was the inoculum used for plant inoculation. Pure culture of *T. harzianum* inoculated into a steril petri dishes and allowed to sporulate. Neem cake and poultry manure were applied at the rate of 10g and 20g per planting pot, and at the rate of 10t/ha, 20t/ha per pot respectively. Holes were excavated around the base of the okra plant and 1g of *T. harzianum* spores was used for inoculation. Data were collected on plant height, number of leaves, petiole length, and percentage leaf abscission.
Results: Number of leaves on pot treated with *M. incognita, T. harzianum* and 20 g of neem cake was significantly ($p \leq 0.05$) different from all other pots. Data obtained indicates that after 3 weeks of planting all the treatment combinations had significant ($p \leq 0.05$) impact on the crops grown in pots treated with 10 g of neem cake which was not significantly ($p > 0.05$) different. Data obtained on plant height of crops pots with the treatment combination of ($M_1T_1N_0$, $M_1T_0N_1$, $M_1T_0N_2$, $M_0T_0N_2$, $M_0T_0N_0$, $M_0T_0P_1$ and $M_0T_0P_0$) were significantly ($p \leq 0.05$) different. Results on petiole length grown in pots with treatment combination ($M_1T_1N_2$, $M_0T_1N_1$ and $M_0T_0P_1$) were not significantly ($p > 0.05$) different from the control. Result obtained on amendment with poultry manure revealed that pot treated with 20t/ha of poultry manure had significant ($p \leq 0.05$) impact on plant height.
Conclusion: Based on the data obtained on the growth parameters, it can be concluded that *T. harzianum* and neem cake significantly increased the growth of okra infested with *M. incognita* and the impact was significantly shown on the plant height and petiole length. Number of leaves were significantly different at 3 and 5 weeks after planting. *T. harzianum* has be proven to be of economic value in agriculture both as biocontrol agent and soil nutrient booster.

Key words: *Meliodogyne incognita*; Soil amendment; Nematode; Okra; Neem cake; Poultry manure

1. Introduction

Okra (*Abelmoschus esculentus*(L.) Moench) is a summer vegetable crop grown in many countries. Itbelongs to the family *Malvaceae*, genus *Abelmoschus*Coombes [1].The leaves are 10–20 centimetres (3.9–7.9 in) long and broad, palmately lobed with 5–7 lobes. The flowers are 4–8 centimetres (1.6–3.1 in) in diameter, with five white to yellow petals, often with a red or purple spot at the base of each petal. The young tender fruits of okra are cooked in curries, stewed and used as soups.Okra plays an important role in the human diet by supplying carbohydrate, protein, fats, minerals, vitamins, and bioactive compounds. *Abelmoschusesculentus* is cultivated throughout the tropical and warm temperate regions of the world for its fibrous fruits or pods containing round, white seeds. Cultivation of okra (*Abelmoschus esculents* (L.) Moench) requires optimum temperature range between 21 to 30°C. This is because it is a warm season vegetable crop, and tis range of temperature is requisite for its proper growth and high yield. Okra can be grown in wide range of soil though the most ideal soil type is sandy loam to clay loam which is rich in organic matter and having better drainage facility.

The seed pods rapidly become fibrous and woody and, to be edible as a vegetable, must be harvested when immature, usually within a week after pollinationCoombes [1]. Okra has enormous nutritional as well as medicinal values. The green tender pods contain oxalic acid, thiamine, riboflavin, nicotinic acid and also vitamin A, B and C. Calcium content is very high as compared to other vegetables, which is about 66 mg per 100 g. Pods are excellent source of iodine which is necessary for the resistant against throat diseases like Goiter. Its ripe seeds are roasted, ground and used as a substitute for coffee in some countries. Mature pods and stems containing crude fibre are used in the paper industry. Okra fruits are used in making soups and stews. Hence, the pathogens of okra are equally of economic importance.

Like other crops,okra is susceptible to attack by many insects and pathogens including fungi, viruses, mycoplasmas and nematodes Hussain*et al.*,[2]; Ahmad *et al.*,[3]; Arain*et al.*,[4]; Iqbal *et al.*,[5]; Srivastava *et al.*,[6]. The most widespread and economically important are the root-knot nematodes (*Meloidogyne spp*.). These are plant-parasitic nematodes from the genus *Meloidogyne*. They exist in soil in areas with hot climates or short winters. About 2000 plants worldwide are susceptible to infection by root-knot nematodes and they cause approximately 5% of global crop loss. Root-knot nematode larvae infect plant roots, causing the development of root-knot galls that drain the plant's photosynthase and nutrients. The short life cycle (usually 25 days at 27˚C, though it may takes longer at lower or higher temperatures) enables root-knot nematode populations to survive well in the presence of a suitable host and their populations build up to a maximum as crops reach maturity.

Control of pathogenic organisms infecting okra is important to achieve the objective of improving the yield performance of okra. In recent times, it is common to find the utilization of biocontrol organisms to mitigate the effect of pathogens on crop. This study aims at evaluating the efficacy of the spore concentration of *Trichoderma harzarium*on *Meloidogyne incognita*infecting okra,comparing different levels of concentration of neem cake extract on *Meloidogyne incognita*anddifferent concentrations of poultry manureon the growth performance of okra (*Abelmoschusesculentus*).

1.1 Root-knot nematode

Root-knot nematode(*Meloidogyne species)*are microscopic and parasitic nematodes which can be found in the roots of infected plants. The root knot nematode species, *M. incognita*, is the most widespread and probably the most serious plant parasitic nematode pest of tropical and subtropical regions throughout the world. It occurs as a pest on a very wide range of cropsWendimu [7]. Though *M. incognita* is found on many soil types, damage and yield losses caused are generally more severe on coarse-textured sandy soils. *Meloidogynespecies* are generally intolerant of flooded soil conditions. Amongstplant parasitic nematodes, root knot nematode (*M. incognita and M. Javanica*) leads to extensive damage in okra crop.Infected plants shows signs of nutrient deficiency such as slow or stunted growth, yellowing of the leaves, wilting of the plant and can cause drastic reduction in yield of the plant Asaturova et al. [8]. Heavy infection of older plants causes the plants to wilt unexpectedly and die off early. The infested plants are therefore chlorotic, stunted and unthrifty Archana and Saxena [9].

According to Mitkowsaki and Abawi [10], host plant species also become one of the factors which contribute to the degree of root galling in plants. Crops susceptible to root knot nematode have been reported to include carrots, lettuce, onions, potato, tomato, soybeans and okra. Okra is particularly susceptible to *M. incognita*. Basically, when the nematode feasts on the plant roots, it interrupts the flow of nutrients and water the plant can absorb. This leaves a plant stunted and wilting, with chlorotic or pale green leaves and culminates in reduced yields. These are above ground symptoms. Below ground, the symptoms of an okra with root knot nematodes includes root swelling at infected site and formation of galls.

1.2 Methods of managing root-knot nematode

Although, managementpractices are effective forcontrolling Root-knot nematodes, they are short-term solutions to Root-knot nematode infestations and mostly reduce their populations in the top layer of soil and effect on crop yield since*Meloidogynespecie* have a wide host range. Methods of control include, cultural control, crop rotation, use of resistant plant varieties, soil solarization, use of soil amendments. Amending soil with organic fertilizersand organic amendments such as plant extracts, compost, cotton gin trash, and sawdust may help control root-knot nemtode. Plant extracts from *Eucalyptus citriodora* (eucalyptus), *Tagetes erecta* (marigold), *Azadirachta indica* (neem) are effective against *M. incognita*, *Hoplolaimus* and *Helicotylenchusmulticinctus*Bernard, *et al.* [11].

1.3 Neem cake in nematode management

The neem cake is byproduct from pressing of fruits and kernels of the Neem tree (*Azadirachta indica*). Neem tree is a tropical evergreen tree upto 1000ft tall originally from India and now found throughout the tropics, particularly in the dry regions of Africa. Neem cake can be used as organic manure and typically contain about 4%

Nitrogen, 0.5 % Phosphorus and 0.5% Potassium and smaller quantities of micro nutrients. Organic amendments are environmentally acceptable but the large quantities required per unit area renders the strategy not practical in large scale farming enterprises.Plant parasitic nematodes have been effectively controlled in various parts of the world using plant materials as source of amendment. Amending the soil with commonly available parts and products of neem is also a common method used against Root-knot nematode affecting okra. Neem cake has been traditionally used by farmers for nematode control on Vegetable crops and cardamon in India and Kenya Ahmed and Kopel[12].

Research has confirmed that neem cake and leaf extracts of *Sena alata*has the potential to effectively control nematodes of *Meloidogyne species*[13].Application of various parts of neem (seeds,leaves) on okra, altered the physiology of host plant and developed a strong defensive mechanism of the root against nematodes. The use of neem products stimulated and change the physiology of plant cells and tissue to repel the nematode parasite. Extracts of fresh greens leaves, showed maximum reduction in egg hatching and cause great mortality of juveniles. Powder of fresh seed showed highest plant growth and reduction in root knot formation Resha, *etal*.[14].

1.3 *Trichoderma speciesas*biocontrol agents

Trichoderma species are ubiquitous and saprotrophic microorganisms classified as Ascomycetes Studholme. *et al.* [15]; Sayed, *et al*. [16]. One can isolate these fungi from decomposing wood, soil, and other organic materials from plants. *Trichoderma specie* prevents nematode penetration and improves plant growth. The conidia of *Trichoderma* attach to nematode cuticle or to egg shell and parasitize on them (Sharon *et al.* [17]. In one remarkable research, *Trichoderma viride* was reported to be effective as a biocontrol agent for virus pathogens when inoculated into the roots of virus infected seedlings of cucumber, after three weeks of inoculation, leaves inoculated with the combination of *Trichodrmaviride* and virus inocula showed no symptoms and tested negative to RT-PCR Etim and Okon [18]. *Trichoderma harzianum* or the chemicals (Carbofuran and Carbosulfan) when applied together as a seed treatment and soil application, improved plant growth parameters of okra and reduced the nematode multiplication as compared to when they were applied either as a seed treatment or soil application Kurulkar *etal*. [19].

Annapurna *et al.*,[20] reported that soil application of *T. harzianum* induce defence-related enzymatic activity like peroxidase(PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and total phenol content in tomato against *M. incognita* and as a result improved the plant growth parameters like shoot height, shoot weight, root length, root weight and reduced the nematode multiplication on the tomato and in the soil as compared to the untreated control. According to Tariq [21], application of *Trichoderma harzianum* and *Trichoderma viride*signifiantly increased shoot weight and decreased root weight of tomato. Both the antagonistic fungi causedsignificantreductions in number of galls, egg masses, and eggs per egg mass and reproductive factors of *M. incognita* in a dose dependent manner. The increases or reductions were slightly greater with *T. harzianum* than those with *T. viride*.

According to Wajahat *etal*. [22], it was also observed that, all the concentrations of *Azadirachta indica* when integrated with *Trichoderma harzianum* increased plant height and fresh shoot weight significantly over control. Maximum decrease in root weight was observed where both *Azadirachta indica* and *Trichoderma harzianum* were mixed at the highest concentrationsWajahat, *etal*. [22]. Adding organic amendments to the soil (e.g. chicken litter) can maximize the *Trichoderma* control activity Islam *et al.* [23].

2. METHODOLGY

2.1 Experimental Site

This experimentwas carried out in screen house at the Botanical Garden University of Calabar, and Plant and Ecological Studies Research Laboratory University of Calabar, Calabar between July, 2022 and October, 2022.

2.2 Sources of Experimental materials

The seeds of okra used for this experiment were obtained from the University of Nigeria Nsukka, Enugu state, Nigeria. The variety used was *Clemsonspinelessv*ariety. Pure culture of *T. harzianum* and nematode inoculum was obtained from the Department of Plant and Ecological studies, University of Calabar, Calabar.While Poultry manure was gotten from the animal house, Department of Genetics and Biotechnology, University of Calabar, Calabar.

2.3 Soil Collection and Sterilization

The soil for this research work was collected at the Teaching/ Research farm of the Department of Crop Science, University of Calabar. The soil samples were collected at the depth of 0-15cm using auger. The soil was thoroughly mixed and sterilized by heating in enamel pot to a temperature of 100^0C for 15minutes. The heat sterilized soil was spread out in a shade for 7days to cool before use for planting. Sterilized soils where weighed into perforated plastic buckets at the rate of 5kg per pot.

2.4 Nematode inoculum Preparation

Nematode inoculum preparation was carried out according to Doncaster [24].
Heavily galled roots of okra plants were uprooted from the soil, soil particles adhering to the roots of the plant were removed by gently rinsing in water. The galled roots were put in moistened polythene bags and then taken to the laboratory for inoculum preparation. Galled roots were chopped into small pieces and put into an electric blender. Small quantity of water was added to the galled tissues and blended till slurry. In order to avoid inactivity of the infective nematode larva, the blending was done for five seconds at each interval. The blended substrate was emptied into 1000ml beaker as more water was added and stirred. 30ml of the suspension was so regulated that the suspension contained approximately 5000larva as mean from three counts using a stereomicroscope. This was the inoculum used for plant inoculation.

2.5Sterilization of materials and preparation of Potato Dextrose Agar (PDA)

Different methods of sterilization were used in this research, the dry heat method which involved the use of dry oven at 160^0C for two hours, for sterilizing glass wares (beamer bottles, petri dishes, cornical flask, beaker)and the wet steam method was done using autoclave to sterilize the PDA. Chemical sterilization which involves the use of 3% hypochlorite and 70% ethanol was used to sterilize samples and the work bench.Nineteen grams(19g) of Potato Dextrose Agar (PDA) was weighed and dissolved in 500ml of distilled water in a cornical flask. The medium was then corked with non-absorbent cotton wool and was wrapped with aluminum foil and sterilized in an autoclave at 121^oC for 15minutes. After heating, chloramphenicol was added and mixed properly. After which the PDA solution was allowed to cool and poured into sterile petri-dishes(90mm). The medium was then allowed to solidify before inoculation.

2.6 Sub-culturing and Inoculation of *T. harzianum*

Prepared PDA was poured into petri dishes and pure culture of *T. harzianum* inoculated into the petri dishes using an inoculating loop and allowed to grow and sporulate for 3-4days.

2.7 Inoculation of potted okra plants using spores of *T. harzianum*
Holes were excavated around the base of the okra plant and 1g of *T. harzianum* spores was used to inoculate into the holes and then covered with soil.

2.8Neem cake application

Neem cake was applied at the rate of 10g and 20g per planting pot equivalent to 2 and 4t/ha respectively. The neem cake extract was applied two weeks before planting.

2.11 Poultry manure treatment, application and planting

Collected poultry manure was cured by spreading it under a shade for 7days to release ammonia from the droppings. It was applied at the rate of 10t/ha, 20t/ha equivalent to 30, 60 grams per pots one week before planting. Okra three seeds of okra were planted and monitored till maturity.

2.13 Data Collection

Data were collected at three weeks interval after planting (3WAP) to the ninth week after planting (9WAP).The following growth parameter data were considered:

Plant height - the height of each plant per pot was measured with a meter rule from the ground surface to the top of the shoot and was expressed as average per plant.

Number of leaves - the total number of leaves of each plant in the pot where counted and expressed as average per plant.

Petiole length - petiole length was obtained by measuring the leaves of *A. esculentus* from the leaf bud to the leaf base.

Percentage leaf abscission - this was obtained using the following formulae:

$$\text{Percentage leaf abscission} = \frac{\text{Number of Abscised leaves}}{\text{Total number of leaves}} \times \frac{100}{1}$$

where: number of abscised leaves was obtained by counting the nodes, total number of leaves was obtained by (number of nodes + number of leaves on the plant).

3. RESULTS

3.1 The impact of the *T.harzianum* and Neem cake to control *Meloidogyneincognita* on number of leaves, plant height and petiole.

Results on the investigation of the impact of *T.harzianum* and Neem cake on the control of *M. incognita* comparing with soil amended with neem cake and poultry manure reveals that Neem cake can be considered a valuable biocontrol plant product and when combined with *T.harzianum*could produce excellent control on *M. incognita* (Tables 1, 2, and 3).The results obtained on number of leaves 3WAP shows that pot treated with *M. incognita*, *T. harzianum* and 20 g of neem cake was significantly ($p \leq 0.05$) different from all other pots. Whereas, at 5 weeks after planting all the pots with the different treatment combinations had no significant($p > 0.05$) impact on the leaves except pot treated with *M. incognita*, *T. harzianum* and without the application of neem cake. Meanwhile the number of leaves at 7 weeks after planting showed a remarkable difference with pots ($M_1T_0N_0$, $M_1T_1N_0$, $M_1T_0N_1$, $M_1T_0N_2$, $M_1T_0N_0$, $M_0T_1N_2$, $M_0T_0N_1$, $M_0T_0N_2$ and $M_0T_0P_1$) being significantly different from pot ($M_1T_1N_2$, $M_0T_1N_1$, $M_0T_1N_0$, $M_0T_0N_0$, $M_0T_0P_2$ and $M_0T_0P_0$). However, the combination of ($M_1T_1N_1$, $M_1T_1N_2$, $M_0T_1N_1$, $M_0T_1N_2$, $M_0T_1N_0$, $M_0T_0N_0$, $M_0T_0P_1$, $M_0T_0P_2$ and $M_0T_0P_0$) show significant ($p \leq 0.05$)impact on the number of leaves on the tested plant after 9 weeks of planting as shown in table 1.Data obtained indicates that after 3 weeks of planting all the treatment combinations had significant ($p \leq 0.05$) impact on the crops grown in pots treated with 10 g of neem cake which was not significantly ($p > 0.05$) different. Similarly, all treatment combination had significant impact on the plant height except pots with treatment combination ($M_1T_1N_1$, $M_1T_1N_2$ and $M_0T_0P_2$) which showed no significant ($p > 0.05$) effect on the plant height of the test crop at 5 weeks after planting.

On a contrary, data obtained from plant height of crops after 7 weeks of planting in pots with the treatment combination of ($M_1T_1N_0$, $M_1T_0N_1$, $M_1T_0N_2$, $M_0T_0N_2$, $M_0T_0N_0$, $M_0T_0P_1$ and $M_0T_0P_0$) were significantly ($p \leq 0.05$) different while all other pots with different treatment combination had no significant impact on the plant height of crops obtained after 7 weeks of planting. However, the number of pots with different treatment combination that had significant impact on the plant height was higher after 9 weeks of planting with ($M_1T_1N_0$, $M_1T_0N_1$, $M_1T_0N_2$, $M_1T_0N_0$, $M_0T_1N_0$, $M_0T_0N_1$, $M_0T_0N_2$, $M_0T_0N_0$, $M_0T_0P_2$ and $M_0T_0P_0$) as shown in table 2.

Results on petiole length at 3WAP, 5WAP and 7WAP shows that there was significant ($p \leq 0.05$) impact on the different treatments on the petiole of all the test crop except the un-inoculated crops which serves as control ($M_0T_0N_0$, $M_0T_0P_1$ and $M_0T_0P_0$) at 3 weeks after planting. Meanwhile, at 5 weeks after planting crops grown in pots with the following treatment combination ($M_1T_1N_2$, $M_0T_1N_1$ and $M_0T_0P_1$)

were not significantly ($p > 0.05$) different from the control while all other pots with other treatment combinations show significant impact on the petiole of the test crops. Similarly, the trend of results obtained for 5 weeks after planting was obtained in 7 and 9 weeks after planting though with variation when all pots with different treatment combination had significant impact on the test crop except the pots treated with ($M_0T_1N_1$ and $M_0T_0P_1$) which showed no significant impact on the petiole as shown in table 3.

The result obtained on amendment with poultry manure reveal that pot treated with 20t/ha of poultry manure equivalent to 60grams/pot had significant ($p \leq 0.05$) impact on plant height at 3 weeks after planting on the number of leaves and petiole length at 5WAP. While at 7 WAP, the impact was significant on only petiole length. There was no significant ($p > 0.05$) impact of poultry manure on number of leaves, plant height and petiole length at 9 weeks after planting.

TABLE 1

Mean separation for Number of Leaves for Okra at 3, 5, 7 and 9 weeks after planting

Treatment Combination	**3WAP**	**5WAP**	**7WAP**	**9WAP**
$M_1T_1N_1$	4.0	4.333	3.000	4.0
$M_1T_1N_2$	4.3	5.333	5.333	3.333
$M_1T_1N_0$	4.0	3.333	2.000	2.333
$M_1T_0N_1$	4.0	4.000	3.000	2.667
$M_1T_0N_2$	4.0	4.333	3.000	2.667
$M_1T_0N_0$	4.0	4.333	2.667	1.667
$M_0T_1N_1$	4.0	5.000	5.667	5.333
$M_0T_1N_2$	4.0	4.667	4.000	5.333
$M_0T_1N_0$	4.0	4.333	4.667	3.333
$M_0T_0N_1$	4.0	4.667	4.000	2.000
$M_0T_0N_2$	4.0	4.000	2.667	2.333
$M_0T_0N_0$	4.0	4.333	4.667	3.333
$M_0T_0P_1$	4.0	5.333	6.333	5.333
$M_0T_0P_2$	4.0	5.333	6.000	6.000
$M_0T_0P_0$	4.0	4.333	4.667	3.333
LSD	0.254	1.403	2.133	2.977

M_0 = *Meloidogyne incognita* (control)
M_1 = *Meloidogyne incognita* (5000 Larvae)
T_0 = *Trichoderma harzianum* (control)
T_1 = *Trichoderma harzianum* ($2.4x10^7$spores/ml)
N_0 =Neem unamended (control)
N_1 = Neem at 10g/pot
N_2 = Neem at 20g/pot
P_0 = Poultry manure unamended (Control)
P_1= Poultry manure at 30grams/pot
P_2= Poultry manure at 60grams/pot

TABLE 2

Mean separation for Plant Height of Okra at 3, 5, 7 and 9 weeks after planting

Treatment	**3WAP**	**5WAP**	**7WAP**	**9WAP**

Combination				
$M_1T_1N_1$	8.867	11.533	13.200	17967
$M_1T_1N_2$	10.300	12.433	15.133	19.867
$M_1T_1N_0$	9.333	9.933	10.467	12.167
$M_1T_0N_1$	8.900	10.200	10.933	10.833
$M_1T_0N_2$	9.133	10.033	10.700	12.000
$M_1T_0N_0$	9.067	10.267	11.200	12.900
$M_0T_1N_1$	9.167	11.667	14.100	20.167
$M_0T_1N_2$	8.900	10.000	11.367	17.100
$M_0T_1N_0$	9.800	10.833	11.800	15.233
$M_0T_0N_1$	9.500	10.700	12.500	14.700
$M_0T_0N_2$	9.267	10.500	10.400	12.233
$M_0T_0N_0$	7.967	9.067	10.067	12.567
$M_0T_0P_1$	7.967	14.967	16.133	23.667
$M_0T_0P_2$	9.167	13.167	15.300	24.700
$M_0T_0P_0$	7.967	9.067	10.067	12.567
LSD	1.898	3.203	4.983	8.181

M_0 = *Meloidogyne incognita* (control)
M_1 = *Meloidogyne incognita* (5000 Larvae)
T_0 = *Trichoderma harzianum* (control)
T_1 = *Trichoderma harzianum* ($2.4x10^7$spores/ml)
N_0 =Neem unamended (control)
N_1 = Neem at 10g/pot
N_2 = Neem at 20g/pot
P_0 = Poultry manure unamended (Control)
P_1= Poultry manure at 30grams/pot
P_2= Poultry manure at 60grams/pot

TABLE 3

Mean separation of Petiole Length of Okra at 3, 5, 7 and 9 weeks after planting.

Treatment Combination	**3WAP**	**5WAP**	**7WAP**	**9WAP**
$M_1T_1N_1$	1.867	2.667	2.500	4.200
$M_1T_1N_2$	2.133	4.100	4.100	6.333
$M_1T_1N_0$	1.867	1.733	1.367	1.833
$M_1T_0N_1$	1.833	1.900	2.033	2.633
$M_1T_0N_2$	1.700	2.467	2.100	2.367
$M_1T_0N_0$	1.767	2.300	1.933	2.133
$M_0T_1N_1$	1.967	3.667	4.533	7.233
$M_0T_1N_2$	1.633	2.300	3.500	6.300
$M_0T_1N_0$	1.967	2.467	2.600	3.633
$M_0T_0N_1$	1.767	2.767	2.800	2.400
$M_0T_0N_2$	1.800	2.267	1.767	2.600
$M_0T_0N_0$	1.167	1.833	2.100	2.800
$M_0T_0P_1$	1.167	4.067	4.667	9.567
$M_0T_0P_2$	1.967	5.400	5.967	11.500
$M_0T_0P_0$	1.167	1.833	2.100	2.800

LSD	0.578	2.024	2.325	4.436

M_0 = *Meloidogyne incognita* (control)
M_1 = *Meloidogyne incognita* (5000 Larvae)
T_0 = *Trichoderma harzianum* (control)
T_1 = *Trichoderma harzianum* ($2.4x10^7$spores/ml)
N_0 =Neem unamended (control)
N_1 = Neem at 10g/pot
N_2 = Neem at 20g/pot
P_0 = Poultry manure unamended (Control)
P_1= Poultry manure at 30grams/pot
P_2= Poultry manure at 60grams/pot

3.2 Percentage leaf abscission of okra caused by *M. incognita*

The results of the percentage abscission of leaves on the impact of *M. incognita* on the test crop was prominent at 7 and 9 weeks after planting. Data obtain shows that the highest percentage of abscised leaves was observed on plant treated with *T. harzianum* and Neem cake with 66.4%, followed by ($M_0T_0N_1$ with 62.3%, $M_1T_1N_0$ with 58.6%, $M_0T_0N_2$ with 56.3%, $M_1T_0N_1$ with 52.9% and $M_1T_1N_2$ with 51.2%). All other treatment combination had the percentage leaf abscission below 50% at 7 weeks after planting Figure 1. Meanwhile, at 9 weeks after planting the percentage leave abscission was generally observed to be lower with all the treatment combination having below 50% except the plant grown in the pot treated with *M. incognita*, *T. harzianum* and no neem applied with 50%. However, the following treatment combination ($M_0T_0N_2$ with 42.9%, $M_1T_1N_1$ with 40%, $M_1T_0N_1$ with 40%, and $M_1T_0N_2$ with 40%) recorded above 39% leaf abscission figure 2.

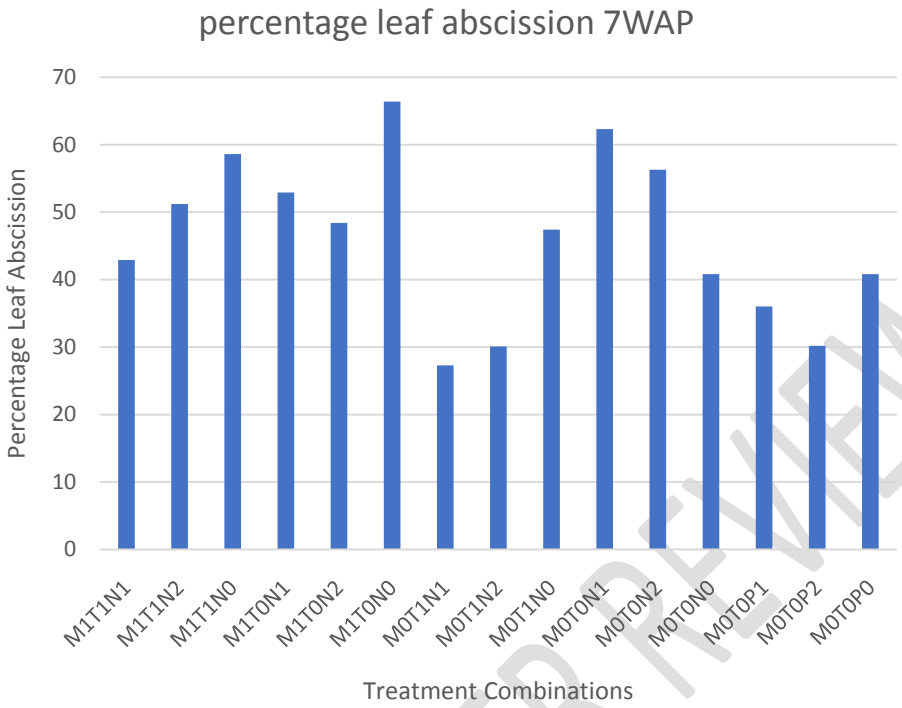

FIG 1:Percentage leaf Abscission of Okra caused infected by *M. incognita* 7 weeks after planting

Key: M_0 = *Meloidogyne incognita* (control)
M_1 = *Meloidogyne incognita* (5000 Larvae)
T_0 = *Trichoderma harzianum* (control)
T_1 = *Trichoderma harzianum* ($2.4x10^7$spores/ml)
N_0 =Neem unamended (control)
N_1 = Neem at 10g/pot
N_2 = Neem at 20g/pot
P_0 = Poultry manure unamended (Control)
P_1= Poultry manure at 30grams/pot
P_2= Poultry manure at 60grams/pot

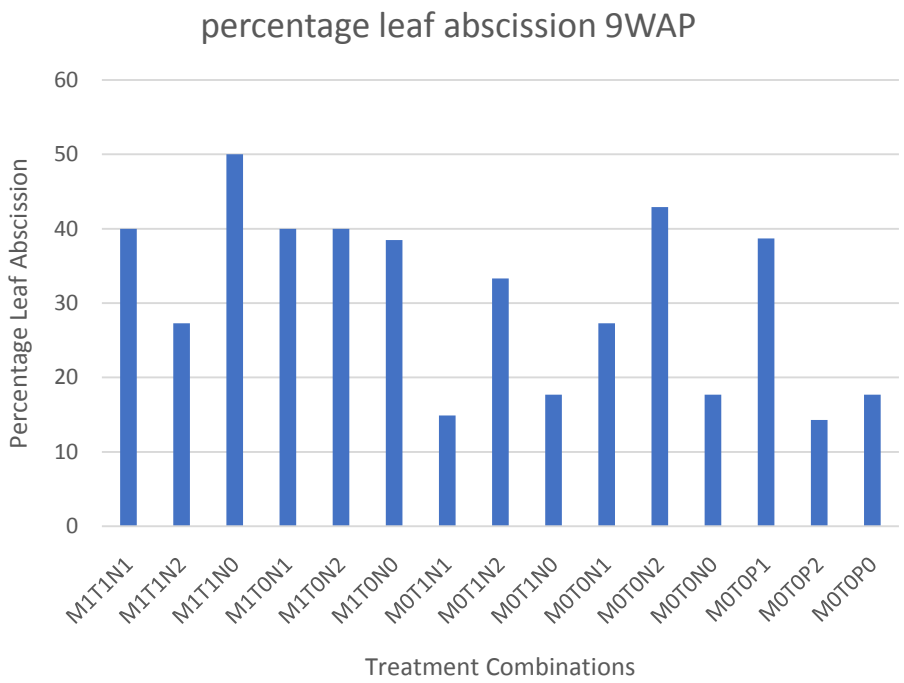

FIG 2: Percentage leaf Abscission of Okra caused infected by *M. incognita* 9 weeks after planting

Key: M_0 = *Meloidogyne incognita* (control)
M_1 = *Meloidogyne incognita* (5000 Larvae)
T_0 =*Trichoderma harzianum* (control)
T_1 = *Trichoderma harzianum* ($2.4x10^7$spores/ml)
N_0 =Neem unamended (control)
N_1 = Neem at 10g/pot
N_2 = Neem at 20g/pot
P_0 = Poultry manure unamended (Control)
P_1= Poultry manure at 30grams/pot
P_2= Poultry manure at 60grams/pot

4. DISCUSSION

The test crop okra, is particularly susceptible to root knot nematode (*M.incognita*). The impact on infected crop varies from stunted growth to wilting, to chlorosis or pale green leaves to leaf abscission and reduced yield. This present study was conducted to investigate the ability of T. harzianum, Neem cake, and poultry manure on okra plant infested with *M. incognita*. From the results, when the growth parameters were assessed, observation reveals that *M. incognita* affected okra significantly, supporting earlier findings of Widmer *etal*. [25] who reported on the impact of *M. incognita* on onions, causing stunting, thicker necks, smaller bulbs and delayed maturity. They also reported that it causes lose in potato by disfiguring the tuber with warty and discoloured skin. They also report symptoms on tomatoes causing patches on fruits St. Amand and Wehner [26].However, with biocontrol agents, the growth parameters were significantly increased. The treatment of soil with *T. harzianum* and neem cake therefore shows great potentials in the enhancement of okra growth parameters.

Data obtained from this research show that pots treated with neem cake at 20 g and *T. harzianum* had significant impact on the number of leaves at 3 weeks after planting. This is in line with the work of Bernard, *etal*. [11] who reported that extracts of *Eucalyptuscitriodoral* (Eucalyptus), *A.indica* (Neem) and *Tageteserecta* (Marigold) were effective against *M. incognita,Hoplolaimusmulticinctus*. Also, Parvertha*etal*.[27] reports that the integration of biological control agents and organic amendment has registered success. However, *T. harzianum* when applied as a single treatment without neem cake had significant impact on plant height and petiole length of the crop at 3 weeks after planting. Similarly, the impact of *T. harzianum* was also observed on plant height and petiole length of crops at 5 weeks after planting. At the later weeks the ability of *T. harzianum* to control *M. incognita* was visible on the number of leaves, plant height and petiole length of crops at 7 and 9 weeks after planting. This result agrees with the results of Sharon *etal*. [17] who published that *Trichoderma* prevent nematode penetration and improve plant growth. Sharon explained that this is done by the conidia of *Trichoderma* attaching to the cuticle of the nematode or to egg shell and parasitizing them. This result conforms to the research of Tariq [21] who reported that *T. harzianum* and *T. viride* significantly increased shoots weight in tomatoes. Furthermore, Harman's reports show that the association of *T. harzianum* and *Bradyrhizobiumjaponicum* to improve the nitrogen utilization capacity Harman [28].

Furthermore, Poveda *etal*. [29] postulates that treatment of soil with*T. harzianum* helps plants to promote disease tolerance or resistance in the host plant. Data from this research reveals that treating soil which is free from *M. incognita* and *T. harzianum* with 10 and 20 g of neem cake was impactful as it had significant effect on plant height and petiole length at 3 and 9 weeks after planting. Whereas, the effect was observed on the number of leaves, plant height and petiole length at 5 weeks after planting. This was not the case at 7 weeks after planting, as pot treated with 10 g had significant impact on number of leaves and petiole length, while pot treated with 20 g of neem cake had significant impact on the number of leaves, plant height and petiole length.

When neem cake at 10 and 20 g was applied on soil with *M. incognita* but without *T. harzianum* results show that the two concentrations of neem cake had significant effect on the plant height and petiole length. This result is in agreement with the outcome of the research by Ahmed and Kopel, 1986 [12] that amending the soil with commonly available parts and products of neem is a common method used against root-knot nematode affecting okra. Ahmed and Kopel also added that neem cake has been traditionally used by farmers for nematode control on vegetable crops and cardamom in India and Kenya. Resha *etal*. [14] in their findings report that extracts of neem stimulated and change the physiology of plant cells and tissue to repel the nematode parasite. They concluded that extracts of fresh green leaves showed maximum reduction in egg hatching and cause great mortality of juveniles while powder of fresh seeds showed highest plant growth and reduction in root-knot formation.

The outcome of this research supports the findings of Rangaswammy*etal*. [30] who evaluated the efficacy of *P. lilacinus* and *T. viride* and botanicals (neem and castor cakes) in suppressing the root-knot nematode, *M. incognita* in tomatoes. Similarly,

Yasmin *etal*. [31] report that extract of neem seed was found to be deadly to the juvenile of *M. javanica* of sweet-gourd compared to the extracts of bark and leaf of neem. Additionally, Wajahat *etal*. [22] reported the integration of *A. indica* and *T. harzianum*increased plant height and fresh shoot weight. Furthermore, Islam *etal*. [23] reports that adding organic amendments to the soil can maximize the control activity of *Trichoderma*.

Data obtained from the crop grown on soil amended with poultry manure reveals that pot treated with 20t/haequivalent to 60grams of poultry manure had significant ($p \leq 0.05$) impact on plant height and petiole length at 3 weeks after planting and on the number of leaves and petiole length at 5 weeks after planting. While for 7 weeks after planting, the impact was significant on only petiole length. However, there was a significant impact of poultry manure on number of leaves, plant height and petiole length of the test plant at 9 weeks after planting. Similar reports is published by many authors including Udo *etal*. [13], Aliyu [32] Adams *etal*. [33] and Olaniyi *etal*. [34] who opined that poultry manure increase fruit nutrient composition, root growth, fruit weight, plant height and yield of okra. Additionally, Emeberi*etal*. [35], Oshunsanya[36] and Ibrahim *etal*. [37] report that organic poultry manure promote fast growth, increase plant height and branches of okra crops.

Furthermore, Smil [38], Makinde *etal*. [39], Prabhu *etal*. [40], and Ayuso *etal*. [41] reported that increased organic manure increased vegetative growth, improved root development and higher yield, activates soil microbial biomass and sustains cropping system through better nutrient recycling. The percentage leaf abscission observed in the results reveals the impact of *M.incognita* on the test plant. It is clear that *T. harzianum* has a significant impact on the growth of Okra evidenced in the Pots treated with *M. incognita* and neem cake but without the application *T. harzianum* ($M_1T_0N_1$ with 52.9%, $M_1T_0N_2$ with 48.4% and $M_1T_0N_0$ with 66.4%). Similarly, crops grown on pot without *M. incognita* and *T. harzianum* but with neem cake had ($M_0T_0N_1$ with 62.3%, $M_0T_0N_2$ with 56.3% and $M_0T_0N_0$ with 40.8%). This can also be concluded from the research that while *T. harzianum* controls and prevent leaf abscission, neem cake promotes and improves plant height and petiole length.

5. CONCLUSION

Based on the data obtained on the growth parameter, that is number of leaves, plant height, and petiole length and percentage leaf abscission. It can generally be concluded that *T. harzianum* and neem cake were effective in the enhancement of okra. The impact was significantly shown on the plant height and petiole length at 3 weeks after planting, although number of leaves were not significantly different at 3 and 5 weeks after planting. *Trichoderma* unlike other soil-borne fungi have be proven in this research to be of economic value in agriculture, not only as biocontrol agent but also to boost soil nutrient. This corroboratingthe works of other authors. Physiologically, reduced, lack and abscission of leaves in plants translates to reduce yield. Hence, the use of *Trichoderma* and neem to control nematode causing leaf abscission is recommended as this research have proven it to be effective in the prevention and control of leaf abscission in okra. Also, the use of *A.indica* and *T.harzianum* in the control of *M. incognita*, was found to prevent environmental degradation, pollution and increased yield of okra.

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