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SUPPRESSION OF Meloidogyne incognita INFECTION ON TOMATO (Lycopersicon esculentum L.) USING NEEM (Azadirachta indica) AND POULTRY MANURE

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ABSTRACT: An experiment was conducted to assess the suppression of root-knot nematode on tomato (Lycopersicon esculentum L.) using neem (Azadirachta indica) and poultry manure. The experiment was conducted at the screen house of the National Horticultural Research Institute (NIHORT) Idi-Ishin, Ibadan Oyo State. Tomato seedlings, Roma, Cobra, Nadira, Lindo, Kiara, and Panther cultivars are grown in pots filled with steam-sterilized soil. Seedlings were treated with neem extract (Azadirachta indica), poultry manure and a combination of neem and poultry manure which were assigned at 0.4g each to inoculated pots of root-knot nematode (5,000 juveniles of nematodes) which 5ml was applied per tomato stand. The treatments applied in the trials reduced root-knot nematode populations in the soil with corresponding increases in plant height, number of leaves, stem girth and fruit yield over the control. There was a significant $(P \le 0.05)$ reduction in the root galls from the treated pots with maximum nematode eggs (1200.00) and juveniles (980.00) observed in untreated pots followed by neem extract application (101.50 eggs) and (720.00 juveniles), poultry manure application (415.00 eggs) and 89.50 juveniles while minimum root-knot nematode eggs of 82.50 and 415.00 juveniles were obtained in neem plus poultry manure. This research recommends the incorporation of poultry manure in nematode-infested soils.

KEYWORDS: *Azadirachta indica, Lycopersicon esculentum,* Poultry manure, Root Knot nematode, Suppression

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INTRODUCTION

Tomato (Lycopersicon esculentum (L). Mill) ranks among other vegetables as one of the most popular and nutritious fruits in the world. The crop belongs to the family Solanaceae, the genus lycopersico. The genus is relatively small within the large and diverse family consisting of about 90 genera (Olaniyan and Ajibola, 2008). Lycopersicon species originated from Mexico. Agriculture began in Central America and later moved to Europe. The tomato plant reaches 1-3 meters (3-10 feet) in height and has a weak stem that often extends to the ground and covers plants with vines. Tomatoes are grown in Nigeria in both wet and dry seasons which attracts higher profits during the dry season due to higher demand than supply. Tomatoes (Lycopersicon esculentum L.) play a vital role in the human diet as a good source of vitamins and minerals. The fruits can be consumed raw or cooked and processed into soup, juice, sauce, ketchup, puree, paste and powder (Olaniyi and Ajibola, 2008). They also serve as an ingredient in stews and vegetable salads. In northern Nigeria, the fruits are mostly chopped and dried for preservation and sale. Lycopersicon esculentum L. requires nutrients such as N.P.K, Mg, Ca, Na and S for good production. These nutrients are specific in function and must be adequately supplied to the plant at the right time and for proper growth and reproduction (Adeyinka and Oyeniyi, 2002). In Nigeria, poor tomato production has been attributed to nematode diseases (Ugonna et al., 2015). Root-knot nematode (Meloidogyne type), being a serious pest of many food crops, especially vegetables, has required studies on organic modification due to the increasing cost of nematicides along with their harmful side effects (Olabiyi, 2004). This will not only improve soil fertility but also serve as a control measure for nematode-inhabiting soils (Ede-Oshemi, 2007). It is believed that the use of compost in controlling the root-knot nematode of Lycopersicon esculentum should enhance the farmer's net profit and production and reduce the production cost. Natural plant products are nowadays, the focus of research efforts due to their potential to be environmental and less harmful but effective chemicals (Das et al, 2010). Plant extracts including aqueous extract of African marigold, basil leaves, and poppy weeds, have been used to test their nematicidal effect in controlling root-knot nematodes (Adepoju et al. 2017). Neem (composted and non-decomposed), rice, straw, leaves, sawdust and poultry manure have been used as an organic amendment in the control of nematode infection on tomato roots and young cuttings (Huang et al, 2015). Therefore, the present research work was conducted to evaluate the nematotoxic effect of neem (Azaidiractha indica) and poultry manure to suppress Meloidogyne incognita infection on Lycopersicon esculentum L. which is economical and safer compared to the chemical control method using with the aim of improving tomato production and yield in nematode infested soil.

MATERIALS AND METHODS

Study Area: The experiment was carried out at the National Horticultural Research Institute (NIHORT), Ibadan Oyo State.

Sterilization of soil and raising of tomato: Top soil was collected and sieved to remove large particles, stones, plastic materials and plant debris. The soil was steam sterilized using a half-cut metal drum source of heat for one hour. The sterilized soil was spread on a large metal sheet after heating and left overnight to cool off before it was used. Five kilograms (5kg) of sterilized soil were placed in 54 plastic pots measuring 10 litres. Seeds of tomatoes which are susceptible to Nematode infection (Roma, Kiana, Nadira, Lindo, Panther and Cobra) were purchased from

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Agro-tropics, Ibadan Oyo State. The nursery was raised from obtained susceptible tomato seeds in the sterilized potting mixture. Tomato was nursed separately in a tray filled with sterilized potting mixture and was left for about four (4) weeks to germinate, grow and become suitable seedlings to be transplanted. Two seedlings of Tomato were transplanted in pots filled with the sterilized potting mixture. The pots were frequently watered. After establishment, the plants were thinned down to one healthy stand per pot. The experimental design used was a completely randomized design with three (3) treatments and three (3) replicates.

Extraction of Nematode Eggs: The inoculums were obtained from infested Celosia argentea roots which were collected from the vegetable farm of the institute. The roots were thoroughly washed with clean water, drained and then chopped into pieces of about 3cm with a sharp knife; the eggs of the nematodes were extracted from the infected roots. One hundred gram (100 gram) of chopped roots were placed in a bottle before adding 0.5% sodium hypochlorite solution and then shaken vigorously for four (4) minutes. The sodium hypochlorite solution containing the nematodes eggs and the root debris was quickly poured through a 200μm mesh sieve nested over a 500μm mesh sieve. The residual sodium hypochlorite in the two sieves was rinsed several times by placing them under slowly running top water. The eggs were collected from the 500μm sieve into a clean beaker and covered as described by (Elbadri et al., 2009).

Root-Knot Nematode Eggs Counting: Nematode eggs were counted using a counting disk. The egg suspension was withdrawn with a syringe. An aliquot suspension of 1ml was withdrawn into the counting dish. A counter and a stereomicroscope were used to count the eggs. The count was repeated two more times to ensure accuracy. The total number of eggs in 1ml was multiplied by the total egg suspension to obtain the total number of eggs in the suspension.

Preparation of Neem and Poultry Manure: Fresh leaves of Neem (*Azadirachta indica*) was placed into the Nylon and then shed dried. After two weeks, it was then ground into powdered form by removing the feathers and shed dried. The prepared Neem and poultry manure were applied as a treatment to the potted experiment for the control aspect using on tomato cultivar (panther) which is highly susceptible to nematodes among the six cultivars screened. The treatment was applied to the twenty (20 pots filled with sterilized soil before transplanting tomato seedlings. The treatment level were; Okg/ha (control), 400kg N.E/ha, 400kg pm/ha, 400kg N+pm/ha and 400kg N+pm (control)/ ha. Each treatment level was replicated four (4) times making a total of 20 pots. The quantity of treatment that was applied to a pot containing 10kg sterilized soil (quantity of soil per pot) will be,

400 kg N.A/ha (4 pots) = 400/1000 = 0.4 g/pot

400 kg (N.E) /ha (4pots) = 400/1000 = 0.4 g/pot

400 kg (N+pm)/ha (4pots) = 400/1000 = 0.4 g/pot

(Neem = 0.2g and poultry manure = 0.2g)

Extraction of Nematode Eggs from Tomato Roots: After termination of the experiment, infected tomato plants were uprooted and the root galls were separated from the shoots. The root galls were washed to remove the soil particles and then thoroughly washed with clean water, drained and then chopped into pieces of about 3cm with a sharp knife; Nematodes eggs were extracted from the infested roots. One hundred gram (100 gram) of chopped roots were

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placed in a bottle before adding 0.5% sodium hypochlorite solution and then shaken vigorously for four (4) minutes. The sodium hypochlorite solution containing the nematodes eggs and the roots debris was quickly poured through a $200\mu m$ mesh sieve nested over a $500\mu m$ mesh sieve. The residual sodium hypochlorite in the two sieves was rinsed several times by placing them under slowly running top water. The eggs were collected from the $500\mu m$ sieve into a clean beaker and covered.

Extraction of Juveniles from The Infected Soil: 100 grams of soil were sampled from infected soil using the sieving and decanting method of nematode extraction to separate the juveniles' from the soil as described by (Bezooijen, 2006). Plastic sieves with tissue paper which contained soil sampled were placed inside a plastic bowl and then 250mls of water was gently poured beneath the bowls in order to extract the juveniles. The setup was left for 48 hours. After which a counter and a stereomicroscope were used to count the juveniles. The count was repeated two more times to ensure accuracy.

Data Collection: Data were collected on the following parameters; plant height, the number of leaves, stem girth, the number of branches, flowers, fruits, Nematode population in the soil, fruit weight, fresh shoot weight, dry shoot weight, Nematode population in the soil and gall index was determined by uprooting each plant gently, washing in a bucket and the dipping water contained in a beaker and then observing through the beaker.

Statistical Analysis: The data obtained were subjected to analysis of variance (ANOVA) using Gen-stat Statistical Package and then means were separated using Duncan's multiple range test at 5% probability.

RESULT AND DISCUSSION

Table 1 shows the effects of different amendments on the plant height of tomato cultivar panther inoculated with $Meloidogynes\ incognita$. The experimental result showed that there was a significant (p = 0.05) increase in the plant height with age. At two weeks after transplanting (WAT), there was no significant (p = 0.05) difference in plant height of tomatoes with different amendments (Neem extract, poultry manure and neem plus poultry manure). However, between the 4th and 8th WAT, there was a significant (p = 0.05) difference in plant height among the various treatments. Poultry manure was observed to have the highest significantly (p = 0.05) plant height of 109.00cm over other treatments, followed by pots treated with neem plus poultry manure with a plant height of 103.00cm.

The result of the study revealed that plant height at harvest differs significantly (p = 0.05) among treatments with the highest recorded from pots treated with poultry manure. However, the lowest significant (p = 0.05) plant height was obtained from untreated pots (control) with a mean value of 83.25cm.

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Table 1: Effect of Different Amendments on the Plant Height (Cm) of Tomato CV. Panther Inoculated with *Meloidogyne incognita*.

TRT	PH1	PH2	PH3	PH4	PH5	PH6	PH7	PH8
T0	36.50ab	44.10c	56.35c	65.93bc	72.38bc	79.20bc	83.00ab	83.25bc
T1	30.95c	44.43c	59.73bc	71.78bc	80.35bc	89.75ab	91.25ab	91.50ab
T2	38.56ab	53.75ab	73.35a	76.50bc	92.73a	100.50a	102.75a	109.00a
T3	39.73a	55.75a	70.08ab	83.75a	85.75ab	89.50ab	96.50ab	103.00ab
T4	32.18bc	44.95bc	61.18bc	69.10bc	69.55c	71.00c	77.75c	86.00bc

Means having the same letter along the columns indicate no significant difference using Duncan's Multiple Range Test at a 5% probability level (p = 0.05). TRT: Treatment, PH: Plant height, T0: No amendment (control), T1: Neem extract, T2: Poultry manure, T3: Neem and Poultry manure, T4: Neem and Poultry manure (control).

Table 2 shows the effects of different amendments (neem extract, poultry manure and neem plus poultry manure) on the number of leaves of the tomato cultivar panther inoculated with *Meloidogyne incognita*. Tomato plants treated with neem extract plus poultry manure were observed to have the highest significant (p = 0.05) number of leaves. The same trend was also observed with tomato plants treated with only poultry manure, while untreated tomato plants were observed with the significantly (p = 0.05) lowest number of leaves per plant.

At one week after transplanting (WAT), tomato plants treated with neem extract plus poultry manure were observed to have the highest number of leaves (9.00) while the lowest significant (p =0.05) number of leaves was obtained from pots treated with neem extract (7.00). The same trend was observed at 2WAT, pots treated with neem extract plus poultry manure were observed with the highest number of leaves (10.50), follows by tomato with the neem extract plus poultry manure (control) with the mean value of 10.25, while the lowest significant (p =0.05) number of leaves were observed from pots of untreated tomato plants (control) with the mean value of 8.25. At 7WAT, no significant (p = 0.05) difference was observed among the treatments. At 8WAT, untreated plant tomato plants were observed with the significantly (p = 0.05) lowest number of leaves per plant (18.75), while tomato plants treated with neem extract plus poultry manure were observed to have the highest significant (p =0.05) number of leaves (25.75). However, at 8WAT, no significantly (p = 0.05) difference was observed in the number of leaves between plants treated with neem extract and neem extract plus poultry manure with a mean value of 20.50.

Table 3 shows the significant effect of poultry manure and neem extract plus poultry manure on the stem girth of tomato inoculated with *Meloidogynes incognita*. Plants treated with neem plus poultry manure were observed to have the highest significant (P = 0.05) stem girth at harvest, followed by plants treated with only poultry manure. However, a significant (P = 0.05) increase was observed in plants treated with neem plus poultry manure over untreated plants (control). At 2 WAT, tomato plants treated with neem extract were observed to possess the highest significant (P = 0.05) stem girth (0.53cm), while tomato plants treated with neem extract were observed with the lowest significant (P = 0.05) stem girth (0.38cm). At 4th to 8th WAT, plants treated with neem extract plus poultry manure were given the highest significant

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(p = 0.05) stem girth, while at harvest, the least significant (p = 0.05) stem girth was observed in tomato plants treated with neem extract (0.75cm).

Table 2: Effect of Different Amendments on the Number of Leaf of Tomato CV. Panther Inoculated With *Meloidogyne incognita*

TRT	NL1	NL2	NL3	NL4	NL5	NL6	NL7	NL8
T0	7.25bc	8.25ab	10.75c	10.50bc	11.75bc	18.00ab	18.20b	18.75c
T1	7.00c	8.50b	11.25ab	11.75b	13.50b	17.25b	19.75ab	20.50bc
T2	8.00ab	9.50ab	12.25a	13.25a	15.25a	19.25a	20.00ab	22.00ab
T3	9.00a	10.25ab	11.75ab	11.75b	13.75ab	17.25b	19.00ab	20.50bc
T4	8.50ab	10.50a	12.00ab	12.25ab	14.75ab	17.00c	18.25b	25.75a

Means having the same letter along the columns indicate no significant difference using Duncan's Multiple Range Test at 5% probability level (p = 0.05). TRT: Treatment, NL: Number of leaves, T0: No amendment (control), T1: Neem extract, T2: Poultry manure, T3: Neem and Poultry manure, T4: Neem and Poultry manure (control).

Table 3: Effect of Different Amendments on the Stem Girth (Cm) of Tomato CV. Panther Inoculated With *Meloidogyne Incognita*

TRT	SG2	SG3	SG4	SG5	SG6	SG7	SG8
T0	0.43b	0.43c	0.58ab	0.60ab	0.68b	0.75b	0.80ab
T1	0.38c	0.50ab	0.53ab	0.65ab	0.68b	0.72b	0.75b
T2	0.43b	0.58ab	0.68a	0.73a	0.75ab	0.80ab	0.88ab
T3	0.53a	0.63a	0.65ab	0.70ab	0.75ab	0.83ab	0.90ab
T4	0.50ab	0.63a	0.66a	0.73a	0.80a	0.85a	0.96a

Means having the same letter along the columns indicate no significant difference using Duncan's Multiple Range Test at a 5% probability level (p = 0.05). TRT: Treatment, SG: Stem girth, T0: No amendment (control), T1: Neem extract, T2: Poultry manure, T3: Neem and Poultry manure, T4: Neem and Poultry manure (control).

Table 4 shows the effects of different amendments on the yield and multiplication rate and root gall index of tomato cultivar panther inoculated with $Meloidogyne\ incognita$. Treatments of tomato plants with neem extract, poultry manure and neem plus poultry manure suppressed root-knot nematode pest. The growth and yield of tomato plants were significantly (p = 0.05) increased in treated pots as a result of amendments.

Untreated tomato plants (control) had a significantly (p = 0.05) lower number of fruit per plant, and weight of fruit per plant and also observed with increased root-knot nematode population. Tomato plants treated with neem extract, poultry manure, and neem plus poultry manure gave a higher significant (p = 0.05) reduction in root-knot nematode population with a lower multiplication rate as opposed to that of the control experiment. The result also shows that pots treated with amendment had significantly (p = 0.05) lower root gall index than that of untreated control. It could be observed that the neem extract, poultry manure and neem plus poultry manure suppressed root-knot nematode population build-up and also reduced the root galling of tomato cultivar panther. Maximum nematode eggs (1200.00) and juveniles (980.00) were

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observed in untreated pots followed by neem extract application (101.50 eggs) and (720.00 juveniles), poultry manure application (415.00 eggs) and 89.50 juveniles while minimum root-knot nematode eggs of 82.50 and 415.00 juveniles were obtained in neem plus poultry manure.

Table 4: Effect of Different Amendments on the Yield And Root Index of Tomato CV. Panther Inoculated With *Meloidogyne Incognita*

TRT	FSW	DSW	NFT	FTW	RW	JS	EGGS	GI
	(g)	(g)		(g)	(g)			
T0	70.62a	9.88ab	3.34c	19.20b	18.95ab	980.00a	1200.00a	3.25a
T1	53.81c	11.50a	7.13bc	22.16bc	19.92a	720.00ab	101.50b	1.75ab
T2	69.46ab	9.40ab	11.54ab	28.43ab	15.55ab	570.50bc	8.50b	1.25b
T3	56.09bc	7.35c	12.18ab	30.18ab	12.49bc	415.00bc	82.50b	1.25b
T4	58.70bc	7.53bc	15.14a	35.33a	11.85c	0.00c	0.00c	0.00c

Means having the same letter along the columns indicate no significant difference using Duncan's Multiple Range Test at a 5% probability level (p = 0.05). FSW: Fresh shoot weight DSW: Dry shoot weight, NFT: Number of fruits, FTW: Fruits weight, RW: Root Weight, JS: Juvenile in the soil, EGGS: Eggs in the soil, GI: Gall index, T0: No amendment (control), T1: Neem extract, T2: Poultry manure, T3: Neem and Poultry manure, T4: Neem and Poultry manure (control).

Screening of the six tomato cultivars (*Roma*, *Kiana*, *Nadira*, *Lindo*, *Panther and Cobra*) has shown that they were susceptible to root-knot nematodes and reacted differently to different levels of root-knot nematodes inoculation. The occurrence of variation in susceptibility among six tomato varieties to *M. incognita* might be due to genetic differences (Jacquet *et al.*, 2005). Tomato cultivars Panther was the most susceptible, the infestation clearly affects the plant growth through galling, shortening and deforming of the roots and lowers the yield The decrease is possibly due to improper uptake and transport of elements, nutrients and water resulting from nematode infection (Mukesh and Dhirendra, 2019). All treatments used as amendments (neem extract, poultry manure and neem plus poultry manure) were found to increase the growth parameters, shoot weight of tomato, the yield of tomato per plant and significantly reduced root-knot nematode population.

The result of this experiment has clearly shown that neem extract (*Azadirachta indica*), poultry manure and neem plus poultry manure have effective nematicidal potential in the management of root-knot nematodes. However, poultry manure plus neem extract gave a significantly higher number of fruits, and fruit weight and efficiently suppress the nematode population. The result is in accordance which the studies conducted where it was observed that the nematicidal activities of poultry manure, alone or in combination with neem (*Azadirachta indica*) extracts were tested against Meloidogyne javanica (Oka and Pivonia, 2002). Soil application of these amendments or the neem extracts alone did not reduce the root galling index of tomato plants or did so only slightly, but the application of the amendments in combination with the neem extracts reduced root galling significantly. It has also been observed that several parts of neem trees and their extracts are known to exhibit insecticidal, fungicidal and nematicidal activities and many neem-based pesticidal formulations have been developed and marketed (Raguraman

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et al., 2004; Suresh et at., 2004). The result of this research work also conforms with earlier reports on the potential of some composted agro-industrial wastes as a management option for the suppression of plant-parasitic nematodes (Nwanguma, and Awoderu, 2002; Akhtar and Malik, 2000). The effect of organic amendments on the suppression of plant-parasitic nematodes may be due to the enhancement of the indigenous soil microfauna and flora (Oka et al., 2007). On the other hand, reports of biological control of plant-parasitic nematodes by antagonists located in the amending material are scarce and refer mainly to materials very different from those used in this study, such as manures (Agyarko et al., 2005). For example, ammonia produced in the dry cork compost might be involved in nematode suppression since the C:N ratio of cork compost is sufficient for nematode management (Khan, et al. 2008.). Other high-molecular-weight substances, such as tannins or phenolic compounds, were also associated with nematode suppression by the composted dry cork and dry-grape marc residues. Such substances are constituents of neem extract and were regarded as responsible for nematode suppression by other organic amendments (Ojo et al., 2013).

Therefore, poultry manure fortified with neem extract is recommended for the management of root-knot nematode. The neem extract and poultry manure should also be tested against other plant-parasitic nematodes and pests. From this study, it can be stated that root-gall nematode disease on tomato plots can be effectively controlled and good yields are obtained in *M. incognita* infested soils.

CONCLUSION AND RECOMMENDATION

Among the tested varieties of tomato panther with high occurrence of the root-knot nematode were seen to be reduced with the combination of neem and poultry which showed better suppression of root-knot nematode population in the soil considerably, allowing better yield and plant establishment. It is evident from this work that the use of neem with poultry manure is very effective in suppressing plant-parasitic nematodes, especially root-knot nematodes and enhanced maximum crop production. From this study, it can be stated that root-gall nematode disease on tomato plots can be effectively controlled to obtain good yields in *M. incognita*-infested soils.

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