RESEARCH ARTICLE

Nematicidal potential of three plant materials against Root-Knot Nematode (*Meloidogyne incognita*) infestation on Tomato (*Solanum lycopersicum*)

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ABSTRACT

Pot trial experiment was conducted at the College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria between the months of June and October, 2014. The study was designed to determine; the nematicidal effect of leaf powders of *Aspilia africana* (African marigold), *Terminalia catappa* (Indian almond) and *Cymbopogon citratus* (Lemon grass) against nematode infestation on tomato; appropriate time of application of plant materials and to compare the effect of the plant materials with synthetic nematicide (carbofuran). The experiment consisted of six treatments with six replicates, arranged in a Completely Randomized Design. Parameters recorded were number of leaves, plant height, number of fruits, weight of fruits, fresh shoot weight, fresh root weight, number of galls in roots, number of nematodes egg in root and number of nematodes juvenile in soil. Result obtained showed that treatments at 1 and 15 days after inoculation (DAI) significantly reduced nematode population in some parameters than 30 DAI, although in some cases, there were no significant differences between treated and untreated. Thus, different powders significantly increased tomato yield when compared with untreated (nematode alone). However treatments with *Aspilia africana* appeared to be most effective, as it recorded least number of galls irrespective of periods of application, also for eggs in the roots and nematode juvenile in soil. Plants treated with nematicide ranked second in reducing number of galls, eggs in roots and nematode juvenile in soil. *Aspilia africana* performed better than *Terminalia catappa* and *Cymbopogon citratus* leaf powders, suggesting that they could be used in the management of root-knot nematodes in tomato production as alternative to chemical nematicides in view of their environmental hazard and pollution problems.

Keywords: *Aspilia Africana*, *Cymbopogon citrates*, Carbofuran, *Meloidogyne incognita*, *Terminalia catappa*

INTRODUCTION

Tomato (*Solanum lycopersicum*) is a member of the family *Solanaceae*, as noted by Tindall (2000). It is indigenous to South America, used as food in Mexico and it is grown all over the world following the Spanish colonization of the America (Jones, 2012). The crop has many varieties that are widely grown all over the world, about 100 genera and more than 2,800 species (Griffin and Lin, 2000). They are dicot plants, some have compound leaves while others have simple leaves (Hahn and Fetzer, 2009).

It is the world's largest vegetable crop after Irish potato (*Solanium tuberosum*), but it tops the list of canned vegetables (Fawusi, 1987). In Nigeria, tomato is one of the most important vegetable crop. It is a good condiment in most diets and very cheap source of vitamins A, C and E (Saltveit, 2003). They contain large quantity of water, calcium and Niacin, all of which are very important in the metabolic activities of man. They also contain carotene and lycopene that help in preventing prostate cancer (Mourvaki *et al.,* 2005).
Tomatoes are planted at an estimated rate of 85% each year and produced more in dry season (Allen, 2008). Consumption is in diverse ways, it can be eaten raw, added as ingredient in many dishes, drank as juice, etc. It is cultivated as a major commercial crop in most countries including Nigeria as revealed by Mahovic *et al*., (2004) though it's cultivation is not without limitation, and one of it is disease infestation caused by plant-parasitic nematodes, particularly root-knot nematodes (*Meloidogyne incognita*). This has become the major limiting factor to profitable tomato production (Ononuju, 1999). The nematodes burrow into the soft tissues of roots tips and young roots and cause the nearby root cells to divide and enlarge. Thus infected plants show symptoms like stunted growth, yellowing of the leaves, wilting, and collapse of individual plants swelling or gall on the roots. All root-knot galls damage the vascular tissues of roots and thus interfere with the normal movement of water and nutrient throughout the plants (Olson, 2004). Nematodes generally are regarded as silent enemies, they have caused losses of up to 80% in vegetable fields where their infestation is very high (Kaskavalci, 2007).

Thus goals of controlling nematode is to manage their population, reducing their numbers below damaging levels (Singh and Prasad, 2011). They may be prevented by avoiding the introduction of the pathogen into the field, discarding of any transplants showing swelling or galling of the root. Other ways or methods whereby nematodes can be controlled include cultural, chemical and biological. Farmers generally have relied on the use of synthetic nematicides over the years and this has resulted to its excessive and unsafe usage (Taniwiryono *et al.,* 2007). Indiscriminate use of synthetic nematicides for the control of nematodes leads to phytotoxicity, environmental pollution and nematodes resistance (Yudelman *et al.,* 1998). On the other hand, its unsafe usage may result in poisoning of humans especially in developing countries like Nigeria (Conway, 1995). To this effect, it is of economic importance to find alternative control strategies which are as effective as synthetic nematicides, safer to farmers, consumers environment and relatively easily available at low price (Fernandez *et al.,* 2001). Such alternatives are the use of biopesticides (nematicides of plant origin) because they are ecological friendly (Javed *et al.,* 2006). Ononuju and Okoye (2003) reported the advantages and potential use of active ingredients of higher plants in controlling plant diseases. Numerous plant species belonging to 57 families have been identified locally to contain nematicidal compounds (Sukul, 1992). They are applied either as soil drench, root dip or as foliar spray (Agbenin, 2004).

The objectives are therefore to;

- determine the nematicidal effects of leaf powders of *Aspilia africana* (African marigold), *Terminalia catappa (*Indian almond*)* and *Cymbopogon citratus* (Lemon grass) on nematode infestation on tomato,
- determine appropriate time of application of the plant powders,
- compare the effect of the plant powders with the synthetic nematicide (Carbofuran).

MATERIALS AND METHODS

Experimental site

The experiment was carried out in the College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Umudike is located on latitude of $5^0 22'$ North and longitude of $7^0 33'$ East and an altitude of 122m above sea level. It is situated in the rain forest zone of Southeast of Nigeria, with an annual rainfall of 1916mm, temperature 19^0C to 35^0C and a relative humidity of 76% (NRCRI, 2010).

Soil sterilization and packing

Sandy loam soil was sterilized and allowed to cool before filling the experimental poly bags with 15kg of soil.

Source of Seeds

Seeds of tomato variety (Admiral) were obtained from the National Horticultural Research Institute (NIHORT) Okigwe Sub-Station Imo State, Nigeria.

Extraction of Nematode Inoculum

Root-knot nematode eggs were extracted from the heavily galled roots of *Celosia argentea* (Soko) using sodium hypochlorite (NaOCl) techniques (Hussey and Barker, 1973). The number of eggs in the suspension was estimated by counting four samples of a millilitre each using down caster counting dish under stereomicroscope and the average was taken. Each millilitre contained 200 nematodes eggs, hence 5mls of the suspension containing 1,000 nematodes eggs were used to inoculate the plants.

Planting of Seeds

The seeds of tomato were planted in the nursery, four weeks after germination, healthy plants were transplanted one per poly bag filled with 15kg sterilized soil.

Inoculation of Nematode Eggs to the Plant

Two weeks after transplanting, the plants were inoculated by pouring a calculated volume of the suspension containing 1,000 nematode eggs extracted by Hussey and Barker (1973) method near the plant by making a groove around it.

Experimental Design

The experiment was arranged in a Completely Randomized Design (CRD) in an open field platform using poly bags with six treatments replicated six times including the control.

2.8 Treatment application

The six treatments were as follows:

T1 = Nematode eggs + 30g *Aspilia africana* leaf powder

T2 = Nematode eggs + 30g *Terminalia catappa* leaf powder

T3 = Nematode eggs + 30g *Cymbopogon citratus* leaf powder

T4 = Nematode eggs + 0.3g ai synthetic nematicide (carbofuran)

T5 = Untreated control (Nematode only)

T6 = Uninoculated control (Nematode and treatment free)

The application of the treatments were done in three phases:

PHASE 1: Treatments were followed immediately after inoculation (1 DAI) of Root-knot nematode eggs.

PHASE II: Treatments were applied 15 days after inoculation (15 DAI) of the Root-knot nematode eggs.

PHASE III: Treatments were applied 30 days after inoculation (30 DAI) of the Root-knot nematode eggs.

Preparation of Plant Extracts

Fresh plant materials were collected and air-dried before they were blended to powder with hand blender. Then 30g of each of the extracts powder were weighed separately and mixed in the sterilized soil in the poly bag at the appropriate time interval.

Data collection

The following data were collected at the end of the experiment: Number of leaves, plant height (cm), number of fruits, weight of fruits (g), fresh shoot weight (g), fresh root weight (g), number of galls in roots, number of nematode egg in roots and number of nematode juvenile found in soil.

Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) and means were compared using Least Significant Difference (LSD) at 5% probability level ($P \leq 0.05$) by using Genstat Discovery Edition 4 Statistical Package.

RESULTS

The effect of treatments on plant height, number of leaves and fresh shoot weight applied at different periods after inoculation of plants with eggs *of Melodoidogyne incognita* is shown in Table 1. The treatments did not result in significant differences in all the parameters irrespective of the period of application. However, uninoculated and control experiments recorded the highest plant height (58cm, 61cm, and 63cm), number of leaves (20.00, 18.00, and 19.50) and fresh shoot weight (187.80g, 91.10g and 76.25g) at 1, 15, and 30 DAI respectively, while the least parameters were recorded in plants with nematode alone for plant height and number of leaves.

Table 1: Effect of treatments on plant height, number of leaves and shoot weight after 1, 15 and 30 days after inoculation

Treatment	Plant height (cm)			No of leaves			Fresh shoot weight (g)		
	1 DAI	15 DAI	30 DAI	1 DAI	15 DAI	30 DAI	1 DAI	15 DAI	30 DAI
T_1	30.00	35.00	53.00	16.50	18.00	12.50	117.10	68.60	41.65
T_2	43.50	56.50	40.00	15.00	15.00	11.00	97.95	78.40	39.60
T_3	45.00	42.00	59.00	12.01	13.50	12.00	55.75	78.10	42.00
T_4	52.00	59.00	37.00	16.02	14.50	11.50	33.95	67.20	48.35
T_5	22.00	26.00	27.00	5.00	8.50	8.50	43.20	47.30	52.25
T_6	58.00	61.00	63.00	20.00	18.00	19.50	187.8	91.10	76.25
LSD 0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 2 : Effect of treatments on fresh root weight, number of fruits and weight of matured tomato fruit after 1, 15 and 30 days after inoculation.

Treatment	Fresh root weight (g)			Number of fruits			Weight of fruit (g)		
	1 DAI	15 DAI	30 DAI	1 DAI	15 DAI	30 DAI	1 DAI	15 DAI	30 DAI
T_1	6.15	14.24	14.06	12.50	15.00	13.00	72.25	172.40	137.65
T_2	28.90	22.65	15.30	10.50	7.50	8.30	77.60	116.55	94.8
T_3	12.55	18.00	13.85	17.50	14.50	13.50	177.30	168.60	201.4
T_4	13.10	14.30	13.70	14.00	10.50	9.50	167.10	193.10	120.4
T_5	59.45	45.25	55.95	4.50	3.00	2.50	41.95	45.00	34.00
T_6	29.85	20.10	17.10	16.00	15.00	12.50	153.90	141.70	200.8
LSD 0.05	30.23	20.50	16.24	6.32	4.60	NS	70.20	77.00	68.40

Table 2 shows the effect of treatments on fresh root weight, number of fruits and weight of fruits. There were significant differences among the treatments. Although, highest root weights were recorded in untreated plants (nematode alone, 59.45, 45.25, and 55.95g), while lowest root weights were observed in plant treated with *Aspilia africana* at 1 DAI (6.15g) and 15 DAI (14.24g) respectively, while at 30 DAI, the lowest was seen in plant treated with nematicide (13.70). Similar observations were recorded in weight of fruits, all the treatments were significantly different with the highest weight of fruit coming from plant treated with *Cymbopogon citratus* at 1 DAI (177.30g) and 15 DAI (193.10g). While that of 30 DAI was from uninoculated plant (200.8g). The lowest weights of fruit were noticed in untreated plants (nematode alone), 41.95g, 45.00g and 34.00g). On number of fruits, significant differences were recorded at 1 DAI and 15 DAI, with highest number of fruits recorded from uninoculated plants (16.00 and 15.00), and the lowest recorded in plant with nematode alone (4.00 and 3.00). While at 30 DAI, there was no significant difference, though highest fruit was seen in plants treated with *Cymbopogon citratus* (13.50) and lowest in nematode alone (2.50).

The result of treatments on number of galls in roots, nematode eggs in roots and nematodes juvenile in soil are presented in Table 3. From the Table, it is shown that number of galls were significantly higher in plant with nematode alone, thus in all the 3 phases (days of treatment application) plant treated with *Aspila africana* showed no gall (0.00) at 15 DAI. It also recorded the least number of galls at 1 DAI (4.00) while at 30 DAI plants treated with nematicide had the highest number of galls (5.00). Although there were significant differences shown among all the treatments except at 30 DAI. Eggs in the roots of tomato crop treated with *Aspilia africana* showed great reduction of egg population (150) though significantly different from other treatments, except plant with nematode alone at 15 DAI. Plants treated with *Aspilia africana* recorded zero (0) egg population and was significantly different. However at 30 DAI, plants treated with nematicide (carbofuran) had least number of egg population (350), they were all significantly different. Finally, observations on nematode juvenile were significantly reduced in both plant treated with *Aspilia africana* (50.00) and nematicide (50) at 1 DAI. Although *Cymbopogon citratus* and *Terminalia catappa* also reduced nematode population.

Table 3: Effect of treatments on number of galls in root, nematode eggs in roots and nematode larvae found in each tomato pots after 1, 15 and 30 days after inoculation.

Treatment	Number of Galls			Eggs in Roots			Nematode larvae in soil		
	1 DAI	15 DAI	30 DAI	1 DAI	15 DAI	30 DAI	1 DAI	15 DAI	30 DAI
T_1	4.00	0.00	9.50	150	0.00	500	50.00	50.00	50.00
T_2	18.00	40.00	12.50	750	2550	6500	100.00	13.50	50.00
T_3	18.50	24.50	11.50	1000	1250	500	26.00	700	100.00
T_4	14.00	13.50	5.00	1300	950	350	50.00	150	0.00
T_5	132.50	140.00	62.00	8700	5900	2100	1095.0	2600	1250
T_6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD 0.05	40.98	53.68	NS	3,373	3,451	1,653.0	3,735	NS	NS

DISCUSSION

This study has shown that nematode population density had damage symptoms on the crop, indicating that the higher the population density of nematode infestation in the field, the higher the extent of damage on crops. This confirms the findings of Ononuju and Fawole, (2000) who reported that the extent of damage by nematode is influenced by the level of soil nematode infestation and environment factors. The results obtained in this study varied among the different days of treatment application. The crop treated with plant extract showed better growth effects than the untreated crops (Chitwood, 2002). Observation from this Study further revealed that in the absence of root- knot nematode infestation and infection, tomato yield will be better since there would be no damage through gall incidence. This was shown in the poly bag without nematodes which had the best yield.

Aspilia africana treatment improved fruit yield. This means that they reduced nematode population beyond the level at which they would have caused economic damage. This fact was confirmed in the result from the poly bag with nematodes alone which gave the least fruit yield due to nematode population that was high enough to cause economic losses or damage. The superior growth observed in some crops with treatment which is similar to uninoculated crops was due to reduction in a disease effect or control of *Meloidogyne incognita* which in turn promoted growth and yield of tomato (Hoseinpoor and Kargar, 2012). Wafaa and Mahmoud, (2013) reported the importance of various soil amendments with the plant botanical and their impact in reducing nematode population and their build up in soil and consequent increase in crop yield.

The study also indicated that there were no significant differences among the treatments on plant height, number of leaves and fresh shoot weight. Observations on these parameters in this study were similar to that of Trudgill and Philips, (1992) who confirmed that nematode infestation led to wilting and stunted growth. There were no significant differences in number of galls, number of fruits and nematode juvenile in soil, at 30 DAI, because the tomato plants must have been severely damaged by nematodes infestation before applying the control measures, which led to reduction in resistance by the plant. The root-knot nematode *Meloidogyne incognita* has been reported by many workers, as an important root parasite infecting tomato thus reducing yield up to 28-68% (Pakeerathan *et al.,* 2009). Also, the extent of galling on roots with corresponding heavy weight of roots recorded in control experiments with only nematode inoculum is a means of detecting the infestation of *Moloidogyne* species and the damage caused.

Invariably, infestation of nematode galls, egg population and juvenile production were significantly reduced mostly at 1 and 15 DAI, in some cases. This could be that the plants were treated earlier and in addition with the effects of various treatment combinations. The plant powders were highly effective in their ability in reducing root galls, egg mass and population of nematode in soil when compared with the untreated crops (Nematode alone). This suggests that powders of the test plants must have been toxic to eggs and or juveniles of nematodes thus reducing the nematode population density as well as galling. Similar results have been reported by other research workers (Babatola, 1990, Akhatar and Alam 1993, Alem *et al.,* 1994).

The effects of the tested plant extracts varied in their toxicity, *Aspilia africana* was highest in potency, followed by *Cymbopogon ciratus* and the *Terminalia catappa.* This could be due to differences in their chemical composition and concentration of toxic components. Such results have also been reported by Firoza and Magbool (1996) where they used different plant extracts against *Helicotylenchus dihystera* in tomato. *Aspilia africana* was comparable to carbofuran (chemical nematicide) in efficacy which suggests the need for its use as an alternative to synthetic chemicals in tomato production.

CONCLUSIONS AND RECOMMENDATION

The extracts of *Aspilia africana, Cymbopogon citratus* and *Terminalia catappa* were found to have nematicidal properties. However, the treatment *Aspilia africana* was significantly most effective at the different days of treatment application. *Aspilia africana* reduced nematodes infestation at zero (0) level and increased total tomato yield. This can be used effectively for the management of root-knot nematode in edible tomato without use of chemical nematicide in view of their hazardous effects in the environment. It will also help farmers to shift from the concept of control to the concept of management which is a procedure directed to reducing and maintaining the number of plant-parasitic nematodes at non-injurious level.

However, further rates of application of the treatments are needed in both greenhouse and field trials. The appropriate application time should be further evaluated.

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