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NEMATODE POPULATION DYNAMICS AFTER APPLICATIONS OF PLANT EXTRACTS AND TRICHODERMA SPECIES AS SOIL AMENDMENTS IN TOMATO FIELD

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ABSTRACT

Three different plant extracts (*Khaya ivorensis*, *Azadirachta indica* and *Daniella oleifera*) and two *Trichoderma* species (*T. harzianum* and *T. atroviride*), singly and in combination, were assessed on nematode population changes and also in the control of plant parasitic nematodes on tomato under field condition during 2018 and 2019 planting seasons. During each trial, plant parasitic nematodes encountered in the field were *Meloidogyne*, *Pratylenchus*, *Xiphinema* and *Helicotylenchus* species. The experiment was a randomized complete block design with 4 x 3 factorial scheme and five replications. Factor A being plant extracts (*Khaya ivorensis*, *Azadirachta indica*, *Daniella oleifera*) and control, and factor B being bio-control agents (*Trichoderma harzianum*, *T. atroviride*) and control, resulting in 12 treatments. Significant reductions were observed on the population of the plant parasitic nematodes at all treatment levels with neem plant extract, in combination with either *T. harzianum* or *T. atroviride* resulted in the greatest significant decrease. Applications of different plant extracts and *Trichoderma* species, singly and in combination, enhanced the growth and yield of tomato infected with parasitic nematodes.

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INTRODUCTION

The production of tomato in tropical Africa covered a total area of about 300,000 ha with an estimated annual production of 2.3 million tonnes (Van der Vossen *et al.*, 2004). Nigeria is the largest producer in tropical Africa with about 26,000 ha and yielding about 879,000 tonnes of fresh fruits annually (FAO, 2004). The fruit is rich in vitamins A, C, riboflavin, niacin and thiamine as well as some minerals like sodium and potassium (Janes, 2004). Tomatoes can grow well in many soil types, ranging from sandy loam to clay-loam. The soil types must be rich in organic matter with a pH range of 6–7 (Van der Vossen *et al.*, 2004). The poor yield of tomato in Nigeria has been attributed to nematode diseases (Olabiyi, 2005 and Udo *et al.*, 2008 and Ogwulumba *et al.*, 2011).

Tomato plants infested with nematodes usually have galled roots and are very vulnerable to wilt and rot-inducing pathogens (Williamson and Gleason, 2003).

Nematodes are notorious foes of virtually all economic crops; they cause tremendous damage to all crops. The nematodes are widespread agricultural pests found in all regions of the world. There is hardly any crop in the world which is free of nematode attacks (Agrios, 2005). Nematodes involve a reduction in quality and quantity of the crop. For sustainable tomato production, efforts must be made to obtain insights on the presence and distribution of nematode pests associated with major crops (Osei *et al.*, 2012).

Nematode infestations on crops greatly have an impact on the health, quality and yield of the crop. Nematodes parasitize on a large number of plants and over 3000

cultivated and uncultivated plant species are reported to be affected (Agrios, 2005). Nematodes are distributed world-wide over a wide range of geographical conditions of temperate, tropical and subtropical regions of the world. Nematodes are found to be the most abundant primitive multicellular animals on earth.

Numerically, nematodes are dominating organisms on earth and they are between 80 to 90% of all multicellular animals on earth maybe nematodes (Agrios, 2005). The major nematodes associated with tomato crops are *Meloidogyne*, *Xiphinema*, *Rotylenchus*, *Pratylenchus*, *Helicotylenchus*, *Hoplolaimus* (Agrios, 2005). The symptoms caused by these nematodes are stunting, yellowing of plants, lesions, root galls, patching of leaves etc. At the site of infection of *Hoplolaimus* species, many lateral roots may emerge giving the root system a bushy appearance (Jones *et al.*, 2013). The most widespread and economically important nematodes are the root-knot nematodes (*Meloidogyne* spp.). Their life cycle can be completed in 25 days at 27°C, but it can take longer at lower or higher temperatures. The short life cycle enables root-knot nematode population to survive in the presence of a suitable host and their population build up to a maximum as crops attain maturity. Root-knot nematodes infect a wide range of important crops and are particularly damaging the vegetable crops in tropical and subtropical areas of the world (Osman *et al.*, 2012 and Youssef *et al.*, 2012). The infested plants are stunted, chlorotic and unthrifty (Archana and Saxena, 2012). Among over hundreds described species of *Meloidogyne*, the four most commonly occurring species are *Meloidogyne incognita*, *M. javanica*, *M. hapla*, and *M. arenaria*. The species of root-knot nematode attack more than 2000 species of plants including almost all cultivated plants. Root-knot nematodes are reported to cause annual losses in tropics up to 29% in tomato, 24% in potato, 22% in okra, 23% in eggplant, 28% in beans and 25% in pepper (Williamson and Gleason, 2003).

Nematodes of the genus *Xiphinema*, commonly called dagger nematodes, parasitize plants. Many of these nematodes, the majority of which belonging to the *Xiphinema americanum*-group, can transmit viruses to plants during feeding (Gozel *et al.*, 2006). It can cause economic damage and death of host crops through feeding on the roots and also by spreading wilting and viral mosaic diseases (Jones *et al.*, 2013). Some species belonging to the *Xiphinema americanum*-group have been detected on tomato,

pepper, oak, pines, hackberry and citrus (Mokrini *et al.*, 2014). Other hosts include sorghum, cotton, turfgrass and pearl millet (Wick, 2012), sugarcane, legumes, banana, corn, sugar beet, chili pepper (Agrios, 2005), cassava, weeds (Rosa *et al.*, 2014).

The most effective method of nematode disease control is the use of chemical nematicide. However, health hazards and the attendant adverse effects of chemical nematicides on the non-target organisms and the environment are serious constraints. Lately, alternative nematode management has been sought by many researchers to reverse this ugly trend. The exploitation of biological control methods for the integrated management of plant parasitic nematodes using antagonistic organisms is fast gaining wide acceptance in many countries (Hashem and Abo-Elyousr, 2011).

The application of *Trichoderma* species as bio-control agents and/or their metabolites for diseases control is one of the promising ways to reduce the farmers' reliance on synthetic chemicals in crop protection. Application of *Trichoderma* species or their metabolites had been reported to be one of the most effective bio-control bio-pesticides recommended for plant disease protection under organic agriculture (Izuogu *et al.*, 2014). *Trichoderma* is listed both in Europe and USA as a biological pesticide permitted for use in organic farming. *Trichoderma* species have been known for a long period of time as registered commercial products and biological control agents for the control of plant diseases. Aside this is the potential of *Trichoderma* species to increase plant growth and development (Woo *et al.*, 2006). *Trichoderma* species are known to involve in complex interactions with host plants and soil microbes (Ruocco *et al.*, 2009). There are reports that some plant extracts contain some bio-nematicidal compounds that could be widely exploited in the control of plant parasitic nematodes (Olabiyi *et al.*, 2011 and Olabiyi and Ayeni, 2016).

The objectives of this study are to assess the effects of *Khaya ivorensis*, *Azadirachta indica*, *Daniella oleifera*, *Trichoderma harzianum* and *T. atroviride*, singly and in combination, on the nematode population changes and also in the control of parasitic nematodes on tomato under the field condition.

MATERIALS AND METHODS

The experiments were conducted at Teaching and Research Farms, Ladoko Akintola University of Technology, Ogbomoso, Nigeria, 8.1227°N, 4.2436°E,

during 2018 and 2019 planting seasons. During each trial, the field was ploughed and harrowed thoroughly. The experimental field was divided into plots measuring 3.0 m x 3.0 m with alleyways of 1.0 m in-between blocks and 1.5 m in-between plots. There were 16 plants per plot at a spacing of 75 cm x 75 cm and 75 cm in-between the rolls. The experimental was a randomized complete block design with 4 x 3 factorial scheme and five replications. Factor A being plant extracts (*Khaya ivorensis*, *Azadirachta indica*, *Daniella oleifera*) and control, and factor B being bio-control agents (*Trichoderma harzianum*, *T. atroviride*) and control, resulting in 12 treatments. The soil was sandy loam in all the treatments. One kilogram of each of the thoroughly washed and chopped stem bark of *Khaya ivorensis*, *Azadirachta indica* and *Daniella oleifera* were soaked separately in 1 liter of de-ionized water (Olabiyi and Ayeni, 2016). These were then centrifuged and filtered through Whatman No. 1 Filter Paper (Whatman, Bristol, UK).

Pure culture of *Trichoderma harzianum* and *Trichoderma atroviride* were obtained from spent Oyster mushroom substrate cultured on Potato Dextrose Agar in the Autoclave at 15 psi for 30 minutes in the laboratory (Baloyi *et al.*, 2011). Identified pure culture of *Meloidogyne*, *Pratylenchus*, *Xiphinema* and *Helicotylenchus* species were obtained from National Horticultural Research Institute (NIHORT), Ibadan, Nigeria and Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Standardized aliquot quantity of 10 ml distilled water containing 1000 juveniles of each nematode was used for the inoculation per plant using a band method.

Two weeks after planting tomato seedlings (Roma VF variety) of three weeks old, the treatments (10 mL each of plant extract and 1×10^7 spores each of bio-control agents) were applied into the soil, either singly or in combination. The treatments were *Khaya ivorensis*, *Khaya ivorensis* + *Trichoderma harzianum* mixture, *Khaya ivorensis* + *Trichoderma atroviride*; *Azadirachta indica*, *Azadirachta indica* + *Trichoderma harzianum*, *Azadirachta indica* + *Trichoderma atroviride*, *Daniella oleifera*, *Daniella oleifera* + *Trichoderma harzianum*, *Daniella oleifera* + *Trichoderma atroviride*, *Trichoderma harzianum*, *Trichoderma atroviride* and control. Where neither plant extract nor bio-control was applied served as the control.

At planting, just before the application of soil treatment and at harvesting, soil and root samples were collected randomly from the experimental plots, three samples per plot, and the nematode populations in 200 g portions of soil and 10 g root sample were determined using the tray method of Whitehead and Hemming (1965). Nematodes are clarified, differentiated and identified to species level using C.I.H Nematodes description packages, of the sets 1-8 of CAB International UK (Luc *et al.*, 1990). Soil and root nematode extractions, following Baermann tray method of Whitehead and Hemming (1965) and routine Physico-chemical soil analysis, following method of Olabiyi *et al.* (2011) were done in the laboratory.

Data were collected on the population of plant parasitic nematodes, number of leaf, plant height, number of fruit and fruit weight per plant. Data collected were subjected to statistical analysis using Analysis of Variance and, where necessary, treatment means were separated with Duncan's multiple range tests.

RESULTS

The experimental soil contained coarse sand (45.0%), fine sand (31.0%), silt (2.7%), pH (6.7 - 6.8), Organic Carbon (9.5-9.6%) and organic matter (16.4-16.6%). The soil is classified as sandy loam. It was evident that the plant extracts and *Trichoderma* species, applied singly and in combination, reduced the populations of the plant parasitic nematode encountered (negative change), whereas there was a significant increase (positive change) in the population of plant parasitic nematodes in the control (where neither plant extracts nor *Trichoderma* species were applied (Table 1).

$$\text{Percentage nematode change} = \frac{\text{Pop. of nematode in 2019} - \text{Pop. of nematode in 2018}}{\text{Pop. of nematode in 2018}} \times 100$$

The application of plant extracts and *Trichoderma* species singly and in combination, significantly ($p < 0.05$) improved the plant growth (Table 2). The control had least plant height and number of leaves. The plant extract and *Trichoderma* species applied singly and in combination significantly enhanced the fruit number and weight of tomato from the plant parasitic nematodes. The control had the least plant growth (Table 3).

Table 1. Effects of plant extracts and *Trichoderma* species, singly and in combination, on the plant parasitic nematode populations dynamics.

Treatments	<i>Meloidogyne</i> species			<i>Pratylenchus</i> species			<i>Xiphinema</i> species			<i>Helicotylenchus</i> species		
	2018	2019	% Change	2018	2019	% Change	2018	2019	% Change	2018	2019	% Change
<i>Khaya ivorensis</i>	1,300b	1,100a	- 15.4%	151b	106c	- 29.8%	48b	20a	-58.3%	10a	4a	-60.0%
<i>K. ivorensis + T. harzanium</i>	1,628c	1,220a	- 25.1%	120b	102c	- 15%	52b	31b	- 40.4%	18ab	6a	-66.7%
<i>K. ivorensis + T. atroviride</i>	1,432b	1,364b	- 5.0%	128b	100c	- 21.9%	40ab	21a	- 47.5%	15a	6a	-60.7%
<i>Azadirachta indica</i>	1,960d	1,136ab	- 42.0%	94a	76b	- 19.2%	20a	12a	- 40.0%	11a	6a	-45.5%
<i>A. indica+ T. harzanium</i>	1,120a	902a	- 19.5%	56a	24a	- 57.1%	22a	10a	- 54.5%	10a	4a	-60.0%
<i>A. indica+ T. atroviride</i>	1,042a	886a	- 15.0%	88a	42a	- 52.3%	22a	12a	- 45.5%	15a	7a	-53.3%
<i>Daniella oleifera</i>	2,486e	1,447c	- 41.8%	184c	106c	- 42.4%	90d	38b	- 57.8%	22b	10a	-54.5%
<i>D. oleifera + T. harzanium</i>	2,971f	1,872d	- 37.0%	192c	106c	- 44.8%	72c	48c	- 33.3%	15a	8a	-46.7%
<i>D. oleifera + T. atroviride</i>	3,500g	2,264e	- 35.3%	245d	121c	- 50.6%	74c	42c	- 43.2%	12a	6a	-50.0%
<i>Trichoderma harzianum</i>	1,341b	956a	- 28.7%	128b	100c	- 21.9%	21a	9a	- 57.1%	16a	9a	-43.8%
<i>Trichoderma atroviride</i>	1,428b	926a	- 35.2%	110b	104c	- 5.5%	22a	10a	- 54.5%	21b	7a	-66.7%
Control	6,898h	12,486f	+ 81.0%	950e	1,084d	+ 14.1%	1,084e	760d	+ 36.7%	387c	648b	+67.4%

Means followed by the same letters within the same column are not significantly different (Duncan test, P<0.05).

Table 2. Effect of plant extracts and *Trichoderma* species, singly and in combination, on the growth of tomato.

Treatment	Plant height (cm)		Number of leaves	
	2018	2019	2018	2019
<i>Khaya ivorensis</i>	100.0a	123.4a	56a	62a
<i>K. ivorensis + T. harzianum</i>	77.0bcd	83.2c	35b	44c
<i>K. ivorensis + T. atroviride</i>	68.0cd	65.1d	35d	40c
<i>Azadirachta indica</i>	80.0bcd	86.9c	27bc	32cd
<i>A. indica + T. harzianum</i>	88.2ab	96.4bc	28bc	36cd
<i>A. indica + T. atroviride</i>	90.1ab	105.3b	30b	41c
<i>Daniella oleifera</i>	74.2bcd	68.5c	23c	20d
<i>D. oleifera + T. harzianum</i>	85.46ac	92.8bc	25bc	22d
<i>D. oleifera+ T. atroviride</i>	62.8d	77.7cd	28bc	32cd
<i>T. harzianum</i>	93.4ab	116.8b	47ab	53ab
<i>T. atroviride</i>	91.6ab	114.6b	43ab	56ab
Control	44.2e	51.2e	14d	18e
S.E	15.6	18.3	11.5	12.4

Means followed by the same letters within the same column are not significantly different (Duncan test, P<0.05).

S.E: Standard Error

Table 3. Effect of plant extracts and *Trichoderma* species, singly and in combination, on the yield of tomato.

Treatment	Number of fruits		Fruit weight (g)	
	2018	2019	2018	2019
<i>Khaya ivorensis</i>	30a	32a	257.3a	266.1a
<i>K. ivorensis</i> + <i>T. harzianum</i>	22b	24b	198.5b	200.7b
<i>K. ivorensis</i> + <i>T. atroviride</i>	19bc	19bc	118.8c	148.7c
<i>Azadirachta indica</i>	17c	16c	119.1c	146.4c
<i>A. indica</i> + <i>T. harzianum</i>	19bc	20bc	201.6b	206.8b
<i>A. indica</i> + <i>T. atroviride</i>	20bc	23b	176.1b	181.5b
<i>Daniella oleifera</i>	18bc	20bc	170.1b	180.2b
<i>D. oleifera</i> + <i>T. harzianum</i>	19bc	18bc	204.9b	201.6b
<i>D. oleifera</i> + <i>T. atroviride</i>	15d	15d	132.8c	181.4b
<i>T. harzianum</i>	23b	28b	200.4b	203.5b
<i>T. atroviride</i>	21bc	26b	199.6b	198.4b
Control	8e	10e	72.7d	68.9d
S.E	3.75	4.21	28.26	30.46

DISCUSSION

The fields were inoculated and the nematode population dynamics, in 2018 and 2019 planting seasons, just after the application of some plant extracts and *Trichoderma* species as soil amendments in tomato field, were established. The results revealed that plant extracts and *Trichoderma* species that were applied singly and in combination significantly suppressed the soil nematode population build-up and also enhanced the growth and yield of tomato.

Various researches have shown that *Trichoderma* may improve plant establishment, enhance root growth, protect against soil and waterborne pathogens and hence decrease root diseases (Young *et al.*, 2002 and Baloyi *et al.*, 2011). *Trichoderma* is capable of producing secondary metabolites with antibiotic activity. Of recent, *Trichoderma* composted hardwood bark isolates, was reported to produce a metabolite (Harzianic acid) with antifungal and plant growth-promoting activity. *Trichoderma* species have been formulated and used as bio-pesticides, bio-protectants, bio-stimulants and bio-fertilizer on a large variety of crops (Vinale *et al.*, 2009 and Izuogu *et al.*, 2014). As a today, *Trichoderma*-based bio-fungicides are realities in agriculture, with more than 50 registered formulations worldwide. The use of organic amendments had been suggested (Olabiyi and Oladeji, 2014) and recommendation made for the use of plant extracts to control nematodes (Olabiyi and Ayeni, 2016).

The current research established *Meloidogyne*, *Pratylenchus*, *Xiphinema* and *Helicotylenchus* species,

are threats to tomato production in Nigeria. The growth and yield of Tomato grown in nematode infested soil were significantly reduced. This was evident on tomato plants that were treated with plant extracts and *Trichoderma* species and those that were not treated. Root knot nematodes, *Meloidogyne* species (of the family: Heteroderidae) for instance, present great threats to food crop production worldwide (Lopez-Perez *et al.*, 2011). In addition to reducing yields, they cause unpleasant, unappealing, and warty appearances on tubers of yam and related species, and root knot symptoms on the root of susceptible crops (Olabiyi *et al.*, 2013).

CONCLUSION

Applications of different plant extracts and *Trichoderma* species, singly and in combination, enhanced the growth and yield of tomato infected with parasitic nematodes. It becomes imperative therefore to find out the nematicidal properties of the plant extracts and *Trichoderma* species during the further study.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.

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