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Pathogenicity Study and Nematoxic Properties of Some Plant Extracts on the Root-Knot Nematode Pest of Tomato, *Lycopersicon esculentum* (L.) Mill

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Abstract: In the screen house, tomato seedlings, cv. DT 69/257, grown in steam-sterilized soil were inoculated with graded inocula of 5,000; 10,000; 15,000; 20,000 and 25,000 eggs of root-knot nematode, *Meloidogyne incognita.* At inocula levels of 15,000; 20,000 and 25,000 eggs of *M. incognita,* number of leaf per plant, plant height, fruit yield and root galls were significantly reduced. In the field planted with tomato seedlings, aqueous extracts from the roots of marigold, nitta and basil plant were applied to root-knot nematode inoculated soil at four levels of 25,000; 500,000; 750,000 and 1,000,000 ppm concentrations, 10 mL per tomato stand. All the aqueous plant root extracts applied in the trials reduced root-knot nematode populations in the soil with corresponding increases in plant height, plant leaf and fruit yield over the control treatment. Significant reduction of root galls from the treated plots indicated effective root-knot nematode control by the aqueous root extracts.

Key words: Pathogenicity, nematoxic, root-knot nematode, root extracts

INTRODUCTION

Tomato, *Lycopersicon esculentum* (L.) Mill, belongs to the family Solanaceae. It grows well in the tropics, though in humid lowland regions. It is an important tropical fruit vegetable. Average yield of tomato in Nigeria, 9 tons ha^{-1} is below the world average of 20 tons ha^{-1} (FAO, 1998). Poor yield of tomato in Nigeria has been attributed to nematode diseases (Alofe and Somide, 1982; Agbenin *et al*., 2004; Abolusoro *et al*., 2004; Olabiyi, 2005). Root-knot nematodes, *Meloidogyne* species, infection has been reported as one of the limiting factors in tomato cultivation which in some cases result in 90-100% yield loss of the crop. The susceptibility of some tomato cultivars has been extensively reported in Nigeria (Wonang and Akueshi, 1990; Nwanguma and Awoderu, 2002; Olabiyi, 2004). Tomato plants infected with root knot nematodes (*Meloidogyne* species) have been reported to show stunted growth, yield loss and conspicuous root galls (Siddiqui, 2004).

The application of plant extracts in the control of nematode pests on agricultural crops is gradually gaining ground especially under the nascent organic agriculture. Costa *et al.* (2003) reported that *Artemisia vulgaris* rhizome extract inhibited egg hatch, caused second-stage juvenile mortality and reduced root gall on root knot nematode, *Meloidogyne megadora*, infected *Phaseolus vulgaris*. Moreover, *Chrysanthemum coronarium* as organic amendments and green manure was reported to significantly reduced nematode infection on tomato roots and improved plant-top fresh weight both in the greenhouse and in micro plots (Bar-Eyal *et al*., 2006). Shoot and root extracts of *Chamaesyce maculata*, *Euphorbia pulcherima* and *Lantana camara* (Cox *et al*., 2006); *Inula viscosa* leaf extracts (Oka *et al*., 2006) and *Bixa orellana* root bark extract (Oladoye *et al*., 2007) have been reported to control or suppress soil inhabiting plant parasitic nematodes.

Application of nematicides had been the practice by which farmers control nematodes, either on tomato or other agricultural crops, in Nigeria (Nwanguma and Awoderu, 2002; Olabiyi, 2004). But restriction on the importation (Anonymous, 2004) of pesticides, nematicide inclusive, has compelled nematologists in Nigeria and elsewhere worldwide to seek for alternative nematode pest control. One of the outstanding alternative control measures against nematode pests is the application of plant extracts (Hoan and Davide, 1979; Olabiyi, 2004; Oka *et al*., 2006).

This study reports pathogenicity studies on tomato, infected by root-knot nematode, *Meloidogyne incognita* and evaluates the use of aqueous root extracts from marigold (*Tagetes erecta* L.), nitta (*Hyptis suaveolens* Poit) and basil (*Ocimum gratissimum* L.) in the control of root-knot nematode pest of tomato cv. DT 69/257.

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MATERIALS AND METHODS

Pathogenicity study: Pathogenicity study of *Meloidogyne incognita* on tomato cv. DT 69/257 was carried out at the Teaching and Research Farm of the Osun Stata College of Education, Ila-Orangun, Nigeria during 2004 cropping year. Eggs of *M. incognita* were extracted from nematode culture that was maintained (Olabiyi and Ndana, 2003) on cock's comb, *Celosia argentea* L. cv. TLV 8 by using chlorox, following Hussey and Baker (1973) method. Serially graded inocula of approximately 5,000; 10,000; 15,000; 20,000 and 25,000 eggs per pot were applied in the soil around the bases of 14-day tomato seedlings, cv. DT 69/257 in a 10 L steam sterilized soil. Tomato plants that were not inoculated with root knot nematode served as the control. Each level of inoculum was replicated four times and the pots were laid out in a completely randomized design pattern in the screen house with temperature of 28±2°C.

Data were taken on plant height, number of leaf, number and fruit weight. Root gall rating was done after termination of the experiment using a rating scheme of 0-5. Where, 0 = no infection, 1 = 1-5% of roots galled; 2 = 6-25% of roots galled; 3 = 26-50% of roots galled; 4 = 51-75% of roots galled; 5 = 76-100% of roots galled (Sasser *et al*., 1984).

Field study: Field experiment was carried out at the vegetable rotation farm of the Teaching and Research Farm of the Osun State College of Education, Ila-Orangun, Nigeria during May-July 2004 cropping year. Tomato seeds cv. DT 96/257, obtained from National Horticultural Research Institute, Ibadan, were cultured in the nursery for two weeks before immediate transplanting on the field. Three tomato seedlings were transplanted per plot of 1 m × 1 m size. A week after transplanting, the tomato seedlings were thinned down to one healthy seedling per stand. A week after thinning exercise, each of the tomato seedlings was inoculated with approximately 2,500 eggs of root-knot nematode by band method, in order to augment the root-knot nematode present in the experimental site before planting. The roots of marigold, nitta and basil were collected, air dried (7 weeks) and ground into powder. One kilogram of each plant root was soaked (24 h) into 1 L distill water and filtered. The filtrate (1,000,000 ppm) was serially diluted to 250,000; 500,000 and 750,000 ppm concentrations and thereafter applied singly at two weeks after inoculation by side placement at 5 cm into the soil and a centimeter away from the tomato plant. Ten mililiter each of the different concentrations was applied per plant. Tomato plants that were treated with water only served as the control. Each treatment was replicated four times and laid out in a randomized complete block design. Number of leaf, plant height, fruit number, fruit weight and root gall index at final harvest (12 week after planting) were recorded. Soil samples, in zigzag form, were collected on the field within 0-15 cm soil depth two days before the application of treatment and at harvest. After termination of the experiment, the nematodes were isolated by Cobb's sieving and decanting method along with Baermann's funnel technique (Southey, 1986) and assessed. All data collected were subjected to analysis of variance using SAS (1997) package. Differences between the means were partitioned using Turkey Krammer's highest significant difference at 5% probability level (p = 0.05).

RESULTS AND DISCUSSION

Pathogenicity study: Tomato cv. DT/257 is susceptible to root-knot nematode, *M. incognita*. The result of pathogenicity study revealed that *M. incognita* suppressed the growth and yield of tomato (Table 1). It was observed that tomato growth (plant height and number of leaf per plant) was significantly reduced as a result of *M. incognita* attack. The higher the inoculum level, the lower the plant height and number of leaf per plant (Table 1). Concurrently on the yield (fruit number and fruit weight), 100% loss was observed on tomato plant that were inoculated with approximately 25,000 root-knot nematode eggs. At highest inoculum level of approximately 25,000 root knot nematode eggs, it was observed on the field that majority of the plants died and very few tomato plants that survived did not produce flower as a result of serious root-knot nematode infestation. As the level of inocula increase, the fruit

Table 1: Growth, yield and root gall index parameters of tomato cv. DT/257 inoculated with *Meloidogyne incognita* eggs

No. of *M. incognita* eggs	Mean plant height	Mean No. of leaves/plant	Mean fruit No./plant	Mean fruit weight (g)	Root gall index
0 (control)	50.2^{a}	38.5^{a}	20.5^{a}	58.8^{a}	0.0^{a}
5,000	21.4^{b}	25.3^{b}	6.0^{b}	12.7^{b}	1.5^{b}
10,000	18.0^{b}	20.7^{b}	2.1^{c}	5.3^{c}	2.6^{b}
15,000	12.5^{c}	10.1^{c}	1.0^{c}	2.6^{c}	4.2^{c}
20,000	11.4^{c}	8.4^{c}	1.0^{c}	2.2^{c}	4.2^{c}
25,000	10.7^{c}	5.6^{c}	0.0^{c}	0.0^{c}	5.0^{d}

No. with same letter(s) in the column are not significantly different. The means were partitioned using Turkey-Kramer Highest Significant Difference at 5% probability level

Table 2: Effects of different concentrations of marigold, nitta and basil plant root extracts on the growth and yield parameters of tomato cv. DT/257 inoculated with *Meloidogyne incognita* of approximately 2,500 eggs

Aqueous root extract of	Concentration (ppm)	Mean No. of leaves/plant	Mean plant height (cm)	Mean fruit No./plant	Mean fruit weight (g)
Marigold	250,000	22.8^{d}	33.1^{c}	13.1^{b}	54.7^{a}
	500,000	24.0^{d}	33.5^{c}	16.3^{a}	55.8^{a}
	750,000	30.0^{c}	37.3^{b}	16.9^{a}	57.0^{a}
	1,000,000	37.3^{a}	45.0^{a}	17.5^{a}	57.8^{a}
Nitta	250,000	21.1^{d}	30.8^{c}	12.8^{b}	54.8^{a}
	500,000	22.3^{d}	32.5^{c}	15.5^{a}	54.6^{a}
	750,000	26.3^{c}	35.6^{b}	15.8^{a}	54.3^{a}
	1,000,000	33.0^{b}	36.2^{b}	16.3^{a}	56.5^{a}
Basil	250,000	21.0^{d}	31.9^{c}	13.0^{b}	53.0^{a}
	500,000	21.8^{d}	32.7^{c}	16.0^{a}	53.4^{a}
	750,000	24.5^{d}	35.7^{b}	16.5^{a}	53.8^{a}
	1,000,000	24.5^{d}	37.8^{b}	17.8^{a}	55.9^{a}
Control		13.3^{e}	20.7^{d}	6.8^{c}	10.3^{b}

No. with same letter in the column are not significantly different. The means were partitioned using Turkey-Kramer Highest Significant Difference at 5% probability level

Table 3: Effects of different concentrations of marigold, nitta and basil plant root extracts on the nematode, *M. incognita*, multiplication rate and root gall index

Aqueous root extract of	Concentration (ppm)	Initial root-knot nematode population (Pi)	Final root-knot nematode population (Pf)	Multiplication rate (Pf/Pi)	Root gall index
Marigold	250,000	2260	940^{c}	41.59	2.5^{b}
	500,000	2250	670^{b}	29.78	1.7^{a}
	750,000	2257	510^{a}	22.60	1.1^{a}
	1,000,000	2247	420^{a}	18.69	0.7^{a}
Nitta	250,000	2254	960^{c}	42.59	2.8^{b}
	500,000	2259	700^{b}	30.99	2.0^{b}
	750,000	2249	545^{b}	24.23	1.1^{a}
	1,000,000	2250	421^{a}	18.67	0.9^{a}
Basil	250,000	2253	900^{c}	39.95	2.8^{b}
	500,000	2246	720^{b}	32.06	2.2^{b}
	750,000	2248	480^{a}	21.35	1.3^{a}
	1,000,000	2258	485^{a}	21.48	0.8^{a}
Control		2248	3791^{d}	168.64	4.8^{c}

NS = Not Significant, No. with same letter(s) in the column are not significantly different. The means were partitioned using Turkey-Kramer highest significant difference at 5% probability level (SAS, 1997)

weight and number of fruit per tomato plant decrease. It was observed that root gall index increases with increasing inoculum level (Table 1). Abolusoro *et al.* (2004) also reported that tomato cv. Roma V was susceptible to root-knot nematode and that *M. incognita* significantly reduced the growth and yield of tomato cv. Roma V. Olabiyi and Ndana (2003) also reported that *M. incognita* is capable of reducing the growth rate and also caused significantly increased galls on cock's comb, *Celosia argentea*, on the field.

Field study: The result presented on Table 2 shows that each concentration of aqueous extracts of marigold, nitta and basil plant roots suppressed root-knot nematode pest of tomato cv. DT/257. The growth and yield of tomato were significantly reduced in the control experiment as a result of root-knot nematode infection. The untreated tomato plant (control) had significantly low number of leaves per plant, plant height, number of fruit per plant and fruit weight. However, the treated tomato plants had significantly high number of leaves per plant, plant height, number of fruit per plant and fruit weight. Aqueous extracts of cakes and leaves of castor oil have been shown to have some systemic action against the root-knot nematode, *M. incognita* and have significantly increased the growth and yield of tomato grown on *M. incognita* infested soil (Akhtar and Alam, 1990).

The result presented on Table 3 shows that soil treated with aqueous root extracts of marigold, nitta and basil plants had higher reduction in population of *M. incognita* with lower percentage multiplication rate as opposed to that of control experiment. The result in Table 3 also shows that soil treated with aqueous plant root extracts had significantly lower root gall index as against that of untreated control. It could be deduced that the aqueous root extracts of marigold, nitta and basil plants suppressed the population build-up of *M. incognita* and also reduced the root galling of tomato cv. DT/257.

Several recently publications have dealt with pathogenicity study and/or nematoxic properties of some plant extracts on nematode pests of crops. Olabiyi (2004) reported the presence of flavonoids in the roots of marigold and basil plants and saponins in the root of nitta plant. Olabiyi and Oyedunmade (2003) in an experiment carried out to find the nematicidal properties of some Nigerian plants on root-knot nematodes, it was reported that marigold (*Tagetes erecta*), basil (*Ocimum gratissimum*), nitta (*Hyptis suaveolens*) and rattle weed (*Crotalaria retusa*) completely (100%) prevented hatching of root-knot nematode eggs and also destroyed (100%) the root-knot nematode juveniles at concentrations of 10% and above. The presence of nematicidal compounds (flavonoids and saponins) in the test plant root extracts could be responsible for significant reduction on the soil population of *M. incognita* and galls of tomato root (Olabiyi, 2004). The chemical compounds that are present in the applied root extracts might likely be responsible for increased growth and reduced loss of tomato yield under the *M. incognita* infestation.

Artemisia vulgaris ethanol extracts was reported to inhibit egg hatch (50% inhibition by 2.35 mg mL^{-1}) and caused second-stage juvenile mortality (50% lethality at 12 h exposure to 55.67 mg mL^{-1}), both in a dose-dependent manner (Costa *et al*., 2003). Moreover, *Chrysanthemum coronarium*, applied as organic amendments and green manure, significantly reduced nematode infection of tomato roots and improved plant-top fresh weight both in the greenhouse and in the micro plots. But ethanol extracts of *Anthemis pseudocotula*, *Circer pinnatifidum*, *Geranium* sp. and *Triticum eastivum* were not as effective against nematode as *C. coronrium* (Bar-Eyal *et al*., 2006). Aqueous and ethanol extracts of leaves and stem of *Rhizophora mucronata* plant exerted more lethal effect in mortality of juveniles as compared to hatching of juveniles. Also *R. mucronata*, when applied as soil amendments at 0.1, 1 and 5% w/w, significantly increased the germination rate of seeds, shoot length, root length, shoot weight and root weight of mash bean and okra infested with *M. javanica*.

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