Original Article

Botany

Nematicidal Effect of some Plants Extracts on Root-Knot Nematodes (*Meloidogyne incognita*) of Tomato (*Lycopersicon esculentum*)

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ABSTRACT [ENGLISH/ANGLAIS]

Seedlings of the hybrid tomato cultivar (cv.) "Roma king" grown in a sandy-loam soil were inoculated with 500 eggs of root-knot nematodes, Meloidogyne incognita on nursery bed with temperature of 26+20C. Flowering, number of leaf, plant height and fruit yield were significantly reduced ($p<0.05$) in comparison with the treated experiment on the plots. About a 100% loss occurred on number of leaf, fruit and fruit weight; unhealthy plants root galling also increased. The tomato seedlings were then transplanted on m2 plots and ethanol extracts from four (4) plant species: Azadirachta indica leaf, Capsicum annuum fruit, Zingiber officinale rhizome and Parkia biglobosa seed were applied and tested for nematicidal activity at four levels of 250, 500, 750 and 1,000 ppm concentrations, applying 5ml per tomato stand. At 1,000ppm concentrations the extracts applied completely (100%) prevented root-knot juveniles with regular and corresponding increase in the tomato plants over the control treatments.

Keywords: Roma king, Root-knot, Meloidogyne incognita and ethanol extracts

RÉSUMÉ [FRANÇAIS/FRENCH]

Les semis de la variété hybride de tomate (cv.) «roi des Roms» cultivées dans un sol sablo-limoneux ont été inoculés avec 500 œufs de nématodes à galles, Meloidogyne incognita sur la pépinière lit avec une température de 26 20 C. Floraison, nombre de feuilles, hauteur de la plante et la production de fruits ont été significativement réduites ($p <0,05$) en comparaison avec l'expérience traité sur les parcelles. Une perte de 100% a eu lieu le nombre de feuilles, de fruits et de fruits poids; malsain plantes racine grippage a également augmenté. Les plants de tomates ont ensuite été transplantées sur des parcelles m2 et extraits d'éthanol à partir de quatre (4) espèces de plantes: Azadirachta indica feuilles, Capsicum annuum fruits, Zingiber officinale rhizome et Parkia graines de biglobosa été appliqué et testé pour une activité nématicide à quatre niveaux de 250, 500, 750 et 1000 ppm, les concentrations applicables 5ml par tomate se. A des concentrations de 1000 ppm extraits appliquées complètement (100%) ont empêché de nodosités de racines juvéniles avec augmentation régulière et correspondant dans les plants de tomate dans les traitements de contrôle.

Mots-clés: Extraits roi des Roms, cécidogènes, Meloidogyne incognita et de l'éthanol

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INTRODUCTION

Tomato, Lycopersicon esculentum (L.) Mill., belongs to Solanaceae or the night shade family. It is an edible fruit of a vine that grows well as annuals in cold and warm regions [1].

Tomato, through a variety of cultured barriers has crossed from one continent to another and has become one of the world's foremost vegetables crops [2]; [3].

In Nigeria the annual production of tomatoes for 1995/1996 is estimated at 555.630 tons and is expected to rise annually as coupled with the rising population and increased farming activities [4].

Poor yield of tomato in Nigeria has been attributed to nematode diseases [5]; [6] and [7]. Infection of root-knot nematode (Meloidogyne incognita) 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 [8].

Application of chemical nematicides have been found as an effective measure for the control of nematode but due to its high toxic residual effect [9], their non availability, besides the requirement of skilled labor for application

however discourage its use especially by small farmers who produced more than 70% of food crop in Nigeria [10]. Because of the toxicity of these chemicals to beneficial flora and fauna in the soil there is therefore need to develop an alternative nematode control strategies [11]. One of the outstanding alternative control measures against nematode pests is the application of plant extracts [12]. Costa et al. [13] reported that Artemisia vulgaris rhizome extracts inhibited egg hatch and caused second stage juvenile mortality and reduced root gall on root knot nematode, Meloidogyne megadora infected Phaseolus vulgaris. A nematicidal compound isothiocynates of Cruciferae, polyacetylenes in chilli pepper, tethienyl in Targetes and enepentyne from Ambrosia artemisifolia when applied in soil significantly reduced egg masses and root galls in tomatoes and increase shoot to fresh and dry weight [14]; [15].

The present study was undertaken to establish pathogenicity and evaluate the efficacy of extracts of some selected plants in the control of root-knot nematodes infection on tomato plants.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Four plant parts were collected within Sokoto environment and taxonomically identified and authenticated at the Herbarium Section, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto State Nigeria, in October, 2009. Samples deposited at the herbarium have a voucher No. 029. The plants part authenticated include:

- Neem (Azadirachta indica A. Juss) leaf.
- Red-bell pepper (Capsicum annuum L.) fruit.
- Ginger (Zingiber officinale Roscoe) rhizome.
- African locust bean (Parkia biglobosa (Jacq.) R.Br ex G. Don) seed.

These plants were selected based on their use traditionally for medicinal purposes.

Preparation of Crude Extracts for Nematicidal Activity

The plants part used includes: Azadirachta indica leaves, Capsicum annuum fruits, Zingiber officinale rhizones and Parkia biglobosa seeds. Each of the plant part was air dried at the same time in a room. But A. indica leaves were dried within 21 days, Capsicum annuum fruits dried for 46 days, Zingiber officinale rhizomes dried in 49 days and Parkia biglobosa seeds dried within 35 days. Later the parts were separately grounded using mortar, along with pestle and sieved into powder; using 60 mesh screens [16]. The grounded plant parts were passed in 60x60 mesh sized screen made of copper wire of diameter 0.0075 (0.1095mm) holding the heavy particles and the grounded part was sieved into light-soft powder.

The leaf, fruit, rhizome and seed powder were prepared in 500g separately. Each powder was separately soaked in ethanol (500g/2 liters ethanol) for 24 hours. The ethanol extract was filtered using Whatman's filter paper No. 3 The filtrates at one thousand parts per million (1000ppm) serve as the ethanol extract, which was serially diluted to 250, 500 and 750 ppm concentrations and later applied singly at 14 days after inoculation by bare surface placement at 3cm into the soil and 1cm away from the tomato plant 5milliliters (5ml) each of the concentrations was applied per plant and the plants were watered regularly. Tomato plants that were treated with water only served as the control. Each treatment was replicated four (4) times and laid out in a randomized complete block design. Plant height, fruit number, fruit weight and root gall at final harvest were recorded. Plant height was measured with meter rule in centimeter (cm), leaves and fruit were counted with hand and fruit weight was measured using a weighing scale in gram (g). Root gall rating was done after termination of the experiment 86 days after planting using a rating scheme of 0 to 5, where 0 = no infection 1=1 to 2 root gall, 2=3 to 10 root gall, 3=11 to 30 root gall, 4=31 to 100 root gall and 5=more than 100 root gall as described by [17].

Soil sample from both sides were collected on the field within 1 – 6cm soil depth two days before the application of the treatment and at harvest.

Extraction of Root-Knot Nematodes

The nematodes were isolated by Cobb's sieving and decanting process as described by [18]. Active root-knot nematodes were isolated from small sample of soil in the polythene bags and finally pass through sieves, using Cobb's decantation and sieving technique, where soil sample taken to laboratory were transferred from the polythene bags into the buckets; more water was added to the sample. The soil was stirred thoroughly and allowed to settle for about three minutes. This allows heavy particles to settle at the bottom of the bucket. The mixture was then poured through a set of scientific sieve of different mesh sizes serially 60, 80 and 120. The siever was

then filled up in another bucket. To collect the residue, the siever was stand at the edge of a Petri-dish and fitting with its undersides upper most and the residue was collected by utilizing gentle stream of water to wash it into the Petri-dish. This was then taken to dissecting microscope where it was observed and the nematodes founds were extracted with acid dropper along with curved nylon tooth brush and introduced using needles into preserving bottles.

Study Area on Nematicidal Activity

The seedlings of the hybrid tomato cultivar (cv) "Roma king" obtained from the local market in Sokoto were raised in the nursery of the botanical garden in the Department of Biological Sciences Usman Danfodiyo University, Sokoto from the period of October – December, 2009.

The tomato seedlings of 12 day old were put in poly bags containing moist sandy-loamy soil labeled and placed on the nursery beds and watered regularly. The seedlings were raised for three weeks (21days) before immediate transplanting on the plot. After 21days, two tomato seedlings of about 4 inches having 4 -5 leaves were transplanted per plot of 1 meter square (m2) size. One week after the transplant, the tomato seedling were thinned down to one healthy seedling per stand. The tomato seedlings were inoculated with root-knot nematode eggs extracted from the root of an infested tomato root system [19].

The nematode inoculum was prepared using infected tomato root system with heavy egg masses. The root of infected portion tomato plant were washed very well, removing the debris and the infected portion of the root cut into small pieces of 2 – 3cm. The segments of the root were placed in a container. A solution of 0.5% sodium hypochlorite (NaOCl) was added to cover the roots and later stirred for 2 -3 minutes. The suspension was poured through the sieves of the jar from top to bottom which are of 80, 200, 500 mesh sizes. Eggs on 500 mesh sieve were gently washed with slow stream of cold tap water to rinse off residual NaOCl. The eggs were collected from the 500 mesh sieve and put into a glass beaker. Eggs suspension was brought to known volume and determined in milliliter and eggs of the active juveniles were quantified by agitating the egg suspension. 2ml of the suspension was used and transferred to the counting slide. A chamber made of 24 cells placed under the light microscope and the eggs were counted (three counts are made in 4 of the 24 cells) in the counting slide by random selection and number of eggs were counted in average of the three counts [20]. 500 eggs were used to inoculate each tomato seedling on the plot.

Analysis of Data

Data collected were subjected to analysis of variance (ANOVA) using [21] package. Differences between the mean were partitioned using Turkey-Kramer's highest significant difference at 5% (P=0.05) probability level.

RESULTS

Effect of Extracts

In this study, it was observed that each concentration of ethanol extracts from Azadirachta indica leaves, Capsicum annuum fruits, Zingiber officinale rhizomes and Parkia biglobosa seeds reduced the root knot nematode pests of the hybrid tomato cv. "Roma king" (table 1). The progressive growth or yield of the tomato plant was significantly reduced (compared to the control) as seen in table 1 because of infestation by the root-knot nematode, M. incognita. In the 4th and 5th week it was observed that, the number and fruit weight, of the untreated tomato has significantly reduced ($p<0.05$). However, the tomato plants that were treated with the plant extract had a significantly higher number of leaves and fresh fruits per plant; the plant height and fruits weight were also significantly more (table 1). It was also observed in table 1 that, Zingiber officinale rhizomes extracts have less number of leaves at 750 and 1000 ppm concentration level it was also observed that the number of plant parameters in terms of height, leaves, fruit and weight of fruits of plants treated with Parkia biglobosa seeds extract at all concentrations is superior over the rest of the extracts applied (table 1).

Effect of Extracts

In this study, it was observed that each concentration of ethanol extracts from *Azadirachta indica* leaves, *Capsicum annuum* fruits, *Zingiber officinale* rhizomes and *Parkia biglobosa* seeds reduced the root knot nematode pests of the hybrid tomato cv. "Roma king" (table 1). The progressive growth or yield of the tomato plant was significantly reduced (compared to the control) as seen in table 1 because of infestation by the root-knot nematode, M. incognita. In the 4^{th} and 5^{th} week it was observed that, the number and fruit weight, of the untreated tomato has

significantly reduced ($p<0.05$). However, the tomato plants that were treated with the plant extract had a significantly higher number of leaves and fresh fruits per plant; the plant height and fruits weight were also significantly more (table 1). It was also observed in table 1 that, *Zingiber officinale* rhizomes extracts have less number of leaves at 750 and 1000 ppm concentration level it was also observed that the number of plant parameters in terms of height, leaves, fruit and weight of fruits of plants treated with *Parkia biglobosa* seeds extract at all concentrations is superior over the rest of the extracts applied (table 1).

The results, indicated in table 2 shows that soils treated with ethanol extracts of *Azadirachta indica* leaves, *Capsicum annuum* fruits, *Zingiber officinale* rhizomes have a very high reduction of the population of *M. incognita* with low percentages of the nematode multiplication rate (population) than those of the control (untreated) experiment, which occurred in 6th week. But, *Parkia biglobosa* seeds extracts inhibit highest reduction in the population of *M. incognita* with low percentages (23.53, 34.78, 21.43 and 22.54%) of the nematode multiplication rate (table 2). Moreover, the results in table 2 also shows that soil treated with ethanol extracts of plant leaves, red-bell pepper fruits, ginger rhizomes and African locust bean seeds extracts have a significant low root gall as against the untreated control. Similarly, ethanol extracts above 10% of neem leaves, red-bell pepper fruits, ginger rhizome and African locust bean seeds suppressed and reduced (100%) population of root-knot nematodes, *M. incognita* juveniles with marked reductions in egg hatching and root galling of the hybrid tomato, which occurred from the 7th and 8th week. It could also be seen in table 2 that the extracts of *Parkia biglobosa* seeds exhibit the least number of root galling in comparison with the rest of the extracts (table 2).

Table 1: Effect *Azadirachta indica* leaf, *Capsicum annuum* fruit, *Zingiber officinale* rhizome and *Parkia biglobosa* seed extracts on the growth and yield parameters of hybrid tomato cv. "Roma king" inoculated with 500 eggs of *M. incognita*.

Ethanol extracts	Concentration (ppm)	Mean No. of leaves/plant	Mean plant height (cm)	Mean No. of fruit/plant	Mean fruit weight (g)
	250	11.4^{d}	18.1^{e}	8.1^{b}	25.0^{a}
A. indica	500	12.0^{a}	18.5^{e}	8.3^{a}	25.4^{a}
leaf	750	15.0^{c}	19.3^{b}	8.5^{a}	27.0^{a}
	1,000	19.3^{a}	23.6^{a}	9.3^{a}	27.4^{a}
	250	11.1^{d}	14.3^{e}	6.2^{b}	21.8^{a}
C. annuum	500	10.3^{d}	15.5^{e}	7.1^{a}	24.8^{a}
fruit	750	10.0^{c}	15.6^{b}	7.2^{a}	24.3^{a}
	1,000	15.0^{b}	16.2^{b}	8.3^{a}	26.5^{a}
	250	11.0^{d}	15.0^{c}	8.0^{b}	21.0^{a}
Z. officinale	500	10.0^{d}	16.3^{e}	8.5^{a}	23.8^{a}
rhizome	750	8.5^{d}	16.3^{b}	8.3^{a}	23.5^{a}
	1,000	8.5^{c}	16.3^{a}	9.5^{a}	25.8^{b}
	250	10.3^{d}	14.8^{c}	8.0^{c}	20.5^{a}
P. biglobosa	500	9.8^{d}	16.0^{c}	8.6^{c}	22.0^{a}
seed	750	8.3^{d}	16.0^{b}	9.8^{b}	23.0^{b}
	1,000	8.0^{e}	16.2^{b}	0.5^{b}	24.3^{b}
Control		10.3^{e}	14.5^{d}	5.8^{c}	9.5^{c}

Numbers with same letter (s) in the column are not significantly different. The means were partitioned using Turkey-krammer Highest Significant difference at 5% probability level.

Table 2: Effects *Azadirachta indica*, *Capsicum annuum*, *Zingiber officinale* and *Parkia biglobosa* extracts on the root-knot nematodes *M. incognita* multiplication rate and root galls

Ethanol extracts	Conc. ppm	Initial root-knot nematodes population (P_i)	Final root-knot nematodes population (P_f)	Multiplication rate (P_f /P_i)	No. of root galls
A. indica	2 50	150	65^{e}	43.33	2.5^{b}
	500	140	50^{a}	35.71	1.5^{a}
	750	146	41^{a}	28.08	1.0^{a}
	1,000	13	35^{a}	25.36	0.6^{b}
C. annuum	250	141	68^{e}	48.23	2.8^{b}
	500	144	37^{b}	25.70	0.5^{b}
	750	143	40^{b}	27.97	1.1^{a}
	1,000	140	38^{a}	27.14	0.8^{a}
Z. officinale	250	138	68^{c}	43.48	2.6^{b}
	500	139	43^{c}	30.94	1.3^{b}
	750	145	36^{c}	28.83	1.2^{a}
	1,000	148	40^{a}	27.03	0.7^{b}
P. biglobosa	250	136	32^{b}	23.53	0.2^{b}
	500	138	48^{b}	34.78	1.3^{a}
	750	150	30^{b}	21.43	0.1^{a}
	1,000	142	32^{a}	22.54	0.4^{a}
Control		145	236^{d}	162.76	4.5^{d}

Not Significant (NS). Numbers with same letter(s) in the column are not significantly different. The means were partitioned using Turkey-Krammer Highest Significant Difference at 5% probability level, by SAS (1997) Package.

DISCUSSION

The increase in plants parameters such as the height, leaf, fruit and fruit weight suggests that the extracts used in this work have potential nematicidal effect on the root knot nematodes. [22] reported that the test plant extracts of neem, chilli pepper, ginger and garlic on the micro plots suppressed the population *M. javanica* and *M. incognita* in tomato.

Although, the chemical compounds that are present in the plant extracts used in this research were not determined, their presence and effectiveness might be responsible for the increasing plant growth and reduced loss of the tomato cv. yield following the attack of the root-knot nematodes, *M. incognita*. This report is in accordance with [23] who noticed that active ingredient of neem (azadirahtin), pepper (sincocin) and locust bean fruit husk (titerpine) when applied as bare-root dip treatment significantly reduced soil nematodes in tomatoes and increase shoots, leaves and fruits of the plants to fresh and dry weight.

A considerable increase of the plant parameters in fruit number and fruit weight at 1,000 ppm concentration shows that the extracts have toxic effect in suppressing the active juveniles of the nematodes in comparison with the control treatments. [6] reported that aqueous extracts of neem, basil, nitta, pepper, and ginger powders when applied to tomato and mash beans suppressed the population of *M. incognita* at concentrations above 10%.

It has been established in this research that plants treated with ginger extracts at 10% concentrations exhibit small number of leaves which may be attributed to the nematicidal property or active chemical components incorporated in the plant part which happened to be less strong than the rest of the plant parts. [24] have used water extracts of ginger rhizome at 25, 50 and 100% w/w and found significant reduction of *M. incognita* in tomato with increase in plant height and shoot weight.

It was established in this research that the untreated tomato plants develop stunted stems, poor flowering and chlorotic leaves with white patches following the attack of *M. incognita* eggs with significant reduction ($p<0.05$) in

number of leaves, height, with about 100% loss on fruit and fruit weight. Olabiyi [7] reported that tomato cv. Roma V was susceptible to root-knot nematodes and that *M. incognita* significantly reduces the growth and yield of tomato cv. Roma V and [25] reported approximately 75% reduction in tomato yield because of this pathogen. He added that ethanol extracts of roots and leaves of *Targetes*, *Hyptis*, *Capsicum* and *Theobroma* when applied in a 10% w/w concentration at 48 hours exposure in the screen house reduced (100%) noxious population of soil nematodes.

It was established in this work that soil treated with the ethanolic extracts had higher reduction in the population of *M. incognita*, which is opposed to that of control experiment in which untreated plants shows significance difference ($p > 0.005$) with an increased poor fruit set up and swollen root galls. Abulusoro [2] reported that the susceptibility in tomato plants infected with root-knot nematodes (*Meloidogyne* spp.,) show stunted growth, yield loss and conspicuous root galls, but a number of plants are thought to contain biologically active ingredients which when applied in the soil reduced the incidence of plant parasitic nematodes.

In this study also, neem (*Azadirachta indica*), red-bell pepper (*Capsicum annuum*), ginger (*Zingiber officinale*) and African locust bean (*Parkia biglobosa*) completely (100%) prevented attack and hatching of root-knot nematodes eggs and also destroyed (100%) the root-knot nematodes juveniles at 1,000ppm concentrations (at 10% and above). This report is in line with [13] who reported that *Artemisia vulgaris* ethanol extracts inhibited egg hatching (50% inhibition by 2.35 mg m L^{-1}) and caused second stage juvenile mortality (50% at 12 hr exposure to 55.67mg m L^{-1}) both in a dose-dependent manner.

It was established in this research that the ethanol extracts inhibited nematode egg hatching and reduced active juveniles although not completely at all the concentrations tested . Unlike the effects shown in this study, extract of the plant *Rhizophora mucoronata* when applied as soil amendments at 0.1, 1 and 5% w/w rate of seed improve shoot length, root length, shoot weight and root weight of mash bean, tomato and okra infested with *M. javanica* [14].

It was established in the research findings that the *Parkia biglobosa* seeds extract exhibit the highest reduction in the root nematodes with lower number of the nematodes population along with marked reduction in root-knots or galling. This result is in accordance with [26] who noticed that plant such as *Parkia biglobosa*, *Hyptis spicigera* and the like have been reported to control *M. incognita* in tomato plants.

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Nil.

CONFLICT OF INTEREST

No conflicts of interests was declared by authors.