International Journal of Organic Agriculture Research and Development Vol. 2(2010).

Evaluation of sweet orange peel aqueous extract as root knot nematode suppressant

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ABSTRACT

The toxic effect of the aqueous extract of sweet orange (*Citrus sinensis*) to the root knot nematode, *Meloidogyne incognita*, was compared to that of carbofuran (a synthetic nematicide) in the laboratory and in the field during the 2007 and 2008 planting season at Ahmadu Bello University, College of Agriculture, Kabba. The laboratory experiment tested the effect of citrus peel extract and carbofuran on egg hatch and juvenile mortality of the nematode while the field experiment tested the effect of the treatments on soil and tomato root populations of the nematode. Sweet orange peel aqueous extract was applied at 0, 25, 50, 75 and 100% concentrations, while carbofuran was applied at 0, 250, 500, 750 and 1000ppm on the field and in the laboratory on egg hatch inhibition and juvenile mortality of *M. incognita*. The experiment lasted for a period of six months on the field and seven days in the laboratory for each year. The results from the field experiment showed that citrus peel aqueous extract and carbofuran solution brought about significant reduction in nematode multiplication rate and consequent root damage (root gall index) as compared with the untreated control. The higher concentrations (750 and 1000ppm) of sweet orange peel aqueous extract were significantly more effective than lower concentrations of 250 and 500ppm in suppressing nematode population in the soil and root. In the laboratory, citrus peel extract and carbofuran inhibited *Meloidogyne incognita* egg hatch and juvenile survival. The result indicates with respect to all the tested parameters that sweet orange peel aqueous extract is toxic to root-knot nematode and can be incorporated into its control system and as potential raw material for manufacturing organic based nematicide.

KEYWORDS: Peel of sweet orange, *Citrus sinensis,* root-knot nematode, carbofuran, tomato

Received: 12 December 2008
Accepted: 15 August 2010

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INTRODUCTION

Tomato crop is grown both as a perennial and an annual and it is herbaceous in nature. It is one of the most important vegetables in many regions of the world ranking second in importance to potato (Anonymous, 1989). It is a source of vitamins A, C and D. Its nutritional value cannot be over emphasized. Tomato is affected by wide range of pests. Prominent among them are the plant parasitic nematodes of which *Meloidogyne incognita* is identified as wide-spread and very devastating on tomato crop.

The disease cause by this group of nematode is the root knot disease and it's characterized by numerous pronounced swelling or gall on the root of tomato. Infected tomato plant suffers vascular damage and disturbed water and mineral uptake. Above ground symptoms are stunting, chlorosis and early senescence (Richard and Nicola, 1990). The most effective and most rapid control is achieved by the use of chemicals known as nematicides. However, nematicides are expensive and can be hazardous both within the crop and the soil. All these problems have greatly limited the use of chemical nematicide by many farmers in Nigeria (Egunjobi and Onayemi, 1981; Oyedunmade et al., 2009). The search for alternative control measures for root-knot nematode has thus become imperative. Hoan and Davide (1979), Maqbool *et al* (1987) and Alam *et al* (1980) suggested the use of plant extracts. Plant extracts are cheap and are readily available compared with conventional nematicides. They are environmentally safe (Oyedunmade *et al.,* 1995; Zurren and Khan, 1984; Egunjobi and Onayemi, 1981). In an environmentally conscious world plant extract holds promise for their acceptability and use by resource constraint farmers.

Natural plant products are at present in the focus of research efforts because of their ability to produce environmentally less harmful, but efficacious bio-chemical substance (Schmutterer, 1990). This will greatly minimize the use of toxic synthetic chemicals. It is against this background that extract of the peel of sweet orange (*Citrus sinensis*) was tested for its toxicity on root knot nematode, *M. incognita*, since the toxicity of its component to other pests, especially the field insect pests, have been reported by researchers (Abolusoro, 2001; Olaifa *et al*., 1987).

The study was therefore, conducted to (1) assess the effect of citrus peel extract on the egg hatch inhibition and on juvenile survival of *M. incognita* in the laboratory; and (2) investigate the effect of treating the root-knot nematode infested soil with citrus peel extract on the soil and root population of root-knot nematode *M. incognita* and the root damage of tomato growing in the medium.

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MATERIALS AND METHODS

The trial was conducted on a research field with known record of infestation of *Meloidogyne incognita* (Abolusoro, 2005) at Ahmadu Bello University, College of Agriculture Kabba, Kogi State, Nigeria between the month of July and December 2007 and was repeated at the same time in 2008. Tomato (cv. Roma V) seedlings were raised in sterilized nursery soil. The seedlings were transplanted to the permanent field on ridges 3 weeks after planting (3WAP) at a distance of 50 cm on ridges which were 90 cm apart. The experiment was 2 x 5 factorial fitted into randomized complete block design and each was replicated five times. Each plant was inoculated with 2000 eggs of *M. incognita* 6 WAP to increase the soil root knot nematode population of tomato plant to economic injury level. Fresh peel of sweet orange (*C. sinensis*) was collected; air dried and ground into fine powder; weighed on weighing balance and soaked in 1 litre of distilled water for 24 hours. The resultant filtrate was taken as the stock solution of strength 100%. Several dilution were made from the stock solution by adding the appropriate amount of distilled water to obtain 25, 50, 75% concentrations and 100ml of it was applied to each tomato plant at 7 WAP. Distilled water only served as the control (0%). Data were collected on number of nematodes in 200 g soil at planting and harvesting by method of Whitehead and Hemming (1965). The population of root knot nematode juveniles in 5g root was determined by the method described by Hooper (1990). The root damage, otherwise known as gall index, was determined using a rating scale 0-5 (Taylor and Sasser, 1988).

Citrus peel was subjected to phytochemical screening so as to determine the presence of the bioactive chemical components. The presence of flavonoids, tannin, saponins and alkaloids were determined by the method described by Trease and Evans (1989).

All data, except those on bioactive chemical components, were subjected to analysis of variance and means separated by Duncan multiple range test (Duncan, 1955).

LABORATORY EXPERIMENT

Egg hatch test: Eggs of root knot nematode, *Meloidogyne incognita,* were extracted using sodium hypochlorite (Hussey and Barker, 1973) from galled root raised in a pure culture of root knot nematode, *M.incognita,* race 2. One hundred freshly extracted root knot nematode eggs were introduced into each of the 40 Petri-dishes which were arranged in laboratory at room

temperature of 28 ± 2^{0}C. Twenty milliliter (20ml) of each of the concentrations (100, 75, 50 and 25%) of the aqueous extract of the citrus peel was added separately to 100 freshly extracted root knot nematode eggs. Distilled water only (0% extract) served as the control. Every treatment was replicated eight times. Observations were made on egg hatch every 24 hour for 7 consecutive days. This was done by counting the number of second stage juvenile which emerged from egg using a stereomicroscope.

Juvenile mortality test: Ten milliliter of carbofuran solutions at 250, 500, 750 and 1000ppm concentrations and 25, 50, 75 and 100% aqueous extract of citrus peel were introduced into each of another set of 40 Petri-dishes. Distilled water only served as control (0%). Standardized 1ml juvenile suspension containing 100 juveniles of root knot nematode *M. incognita* was introduced into each of the transparent Petri-dishes containing different concentrations of citrus peel extract and carbofuran solution. The contents of the Petri-dishes were incubated at a temperature of 28± 2^{0}C. The Petri-dishes were covered with glass to prevent evaporation. The experimental design in the laboratory was a randomized complete design. Each treatment was replicated 5 times. Counts of dead juveniles were made initially at 6 hours and then 12 hours of setting up the experiment. Thereafter, the observations were made at every 24th hour (daily basis) interval for a period of 7 consecutive days. The juveniles that did not respond to touch of needle were recorded as dead.

RESULTS

Table 1 shows the effect of citrus peel extract and carbofuran on soil and root population of *M incognita* as well as gall index of infected tomato. The result shows that the parameters under study were significantly lower in the carbofuran treatments as compared with the citrus peel aqueous extract. All the treatments reduced nematode population both in the soil and in the tomato root at harvest; and also tomato root damage (gall index) was significantly reduced in all the treatments as compared with the control. The level of reduction of the nematode populations in soil and tomato root, and gall index by carbofuran and citrus peel extract was directly proportional to the quantity of treatment applied.

Table 2 shows the comparative effect of carbofuran and citrus peel extract on egg-hatch inhibition of *Meloidogyne incognita.* The result shows a total egg-hatch inhibition at the higher concentrations of carbofuran and citrus peel extract from Day 1 to Day 7. Egg hatch inhibition was significantly

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higher in all the treatments than the control. While few juvenile emerged at the lower level (25%) of citrus peel extract and 250ppm of carbofuran.

Table 3 shows the effect of carbofuran and citrus peel extract on juvenile mortality of *M incognita* under laboratory condition. The result shows that both carbofuran and citrus peel extract were effective in causing mortality on *M. incognita* juvenile. Mortality was observed at all the concentration levels of carbofuran and citrus peel treatment from 6th hour of treatment application. 100% mortality was observed at all dosage levels of carbofuran 24 hours (Day 1) after treatment application. By day 2 of the experiment, 100% mortality was recorded in all the dosages of citrus peel treatments except at 25% concentration where 96% mortality was observed.

Table 1: Effect of citrus peel extract and carbofuran on soil and root population of M. Incognita as well as gall index Off the infected tomato

Treatment Concentration `	Initial M. Incognita Population (P)		Final M. Incognita Population on (20cm) soil (PF)		Nematode Multiplication rate (%) PF/P1x100		Number o juvenile in 5g root		Gall index	
	2007 trial	2008 trial	2007 trial	2008 trial	2007 trial	2008 trial	2007 trial	2008 trial	2007 trial	2008 trial
C. Sinensis 0	2098	2111	2458.00i	2426.20h	117.1b	1149b	24.67e	26.67e	4.50c	4.25c
25%	2100	2112	969.25h	925.3f	46.11a	43.81a	17.33d	18.33d	3.20b	3.3ab
50%	2097	2101	877.21g	896.51g	41.81a	466.7a	13.67c	13.00c	2.88a	2.90ab
75%	2099	2103	812.50e	784.20e	38.89a	37.29a	12.00c	12.33c	2.97a	2.88a
100%	2112	2110	644.90c	648.21c	30.49a	30.72a	10.67c	10.67b	2.83a	2.83a
Cabofuran 0	2099	2110	2449.90i	2464.80h	129.50b	116.78b	27.00e	29.33e	2.50c	4.25c
250ppm	2112	2114	774.00f	780.22e	36.64a	36.90a	13.00c	16.00c	3.13b	3.38ab
500ppm	2709	2104	66p9.00d	660.0d	31.80a	36.96a	11.67c	12.00c	2.93a	2.88a
750ppm	2099	2097	565.71b	602.0b	26.95a	28.10a	8.00b	11.00b	2.97a	2.88a
1000ppm	2103	2100	466.50a	459.0a	22.18a	21.85a	5.67a	6.67a	2.83a	2.3a
SE	NS	NS	21.94	45.38	8.17	7.35	6.77	6.66	0.17	0.17

`Figures with the same letter (s) in the column do not differ significantly at P<0.05 according to
` Ducan's multiple range test.

Table 2: Comparative effect of carbofuran and citrus peel extracts on egg hatch inhibition of Meloidogyne incognita

		FIRST TRIAL						SECOND TRIAL						
Treatment	Concentration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	7Day	1Day	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
C. Sinensis	25%	1.5a	1.5a	7.0b	9.0b	9.0c	9.0c	9.0e	0a	1.4a	5.0a	7.2b	9.0c	8.5c	9.4
	50%	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a
	75%	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a
	100%	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a
Carbofuran	250ppm	0a	1.5a	1.5a	1.5a	2.0b	2.0b	2.0b	0a	1.2a	1.3a	2.2a	2.2b	2.2b	2.2b
	500%	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a
	750ppm	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a
	1000ppm	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a
Control	0	17.7b	33.0b	38.0c	62.0c	75.5d	91.0d	91.0d	16.9b	35b	41c	60b	79d	93d	93d
SE		1.03	2.12	1.65	1.23	1.44	1.55	0.55	1.04	2.12	1.70	1.23	1.42	0.51	0.55

Figure with the same letter in the column do not differ significantly at P < 0.05 according to Duncan's Multiple range test.

Table 3: Comparative effect of carbofuran and citrus peel extract on juvenile mortality of M. Incognita (J2) under . Laboratory condition

Treatment	Concen-Tration (%)	FIRST TRIAL 6hrs	12hrs	Day1	Day2	Day3	Day4	Day5	Day6	Day 7	SECOND TRIAL 6hrs	12hrs	Day1	Day2	Day3	Day4	Day5	Day6	Day 7
Citrus Sinensis	25%	0f	10.5f	71.0c	96.0b	100a	100a	100a	100a	100a	0e	10.5d	10.5c	100a	100a	100a	100a	100a	100a
	50%	1.0e	19.0e	87.0b	100a	100a	100a	100a	100a	100a	5.7d	19.1cd	72.2b	100a	100a	100a	100a	100a	100a
	75%	10.0d	24.0d	100a	100a	100a	100a	100a	100a	100a	16.0c	25.0c	81.5b	100a	100a	100a	100a	100a	100a
	100%	20.0b	40.5c	100a	100a	100a	100a	100a	100a	100a	20.0b	41.2b	100a	100a	100a	100a	100a	100a	100a
Carbofuran	250ppm	8.0d	27.0d	100a	100a	100a	100a	100a	100a	100a	8.5d	26.1c	100a	100a	100a	100a	100a	100a	100a
	500ppm	16.2c	36.0c	100a	100a	100a	100a	100a	100a	100a	16.3c	37.0b	100a	100a	100a	100a	100a	100a	100a
	750ppm	25.0b	52.5b	100a	100a	100a	100a	100a	100a	100a	16.3c	37.0b	100a	100a	100a	100a	100a	100a	100a
	1000ppm	32.0a	60.0a	100a	100a	100a	100a	100a	100a	100a	34.0a	62.0a	100a	100a	100a	100a	100a	100a	100a
Control	0	0f	0g	0d	0c	0b	3.3b	6.0b	8.5b	10.1b	0e	0e	0d	0b	0b	0b	0b	0b	0b

Figures followed by the same letter(s) in the same column are not significantly different at P<0.05 using Ducan's multiple range test.

Table 4 shows the quantitative bioactive chemical compositions of sweet orange (citrus) peel. The table shows that citrus peel contains flavonoids, saponins and tannin while alkaloid was completely absent.

Table 4: Bio-active chemical composition of citrus peel

Alkaloid	Flavonoids	Saponins	Tannin
Nil	44.00%	9.78%	6.22%

DISCUSSION

The citrus peel extract and carbofuran significantly reduced the soil and tomato root population of the root knot nematode, *M. incognita*. Root damage (gall index) was also significantly reduced, in both treatments, in proportion to the levels of application and was significantly different from the control treatment. This observation agrees with those of earlier researchers. Abid *et al* (1995) and Sellami and Moufarrah (1994) reported the effectiveness of various plant extract used in their various experiments in suppressing both plant root and soil population build up of nematode and consequent reduction in the root damage in the tested crops. The citrus peel extract brought about egg hatch inhibition as well as heavy mortality of nematode juvenile and compared favourably with synthetic nematicide (carbofuran). The observed nematoxic effects of the citrus peel extract can be attributed to the presence of nematicidal chemical components that were seriously injurious to *Meloidogyne incognita* eggs and infective second stage juveniles (J2). The findings from the study corroborate that of earlier researchers like Pandy (1990) and Mani and Al Hinai (1998) who reported the toxicity of various plant extracts used in the experiments.

Saponins, flavonoids and tannin were present in the citrus peel and are probably responsible for the toxicity of the citrus peel extracts to *M. incognita*. This observation agrees with Alam *et al* (1989) who reported the effectiveness of flavonoid, tannin and saponin in reducing *M. incognita* population and enhancing egg-hatch inhibition of *M. incognita* in their various experiments. This type of control on plant parasitic nematode had been reported by many workers including Oyedunmade *et al* (2001), Oyedunmade (2004) and Olabiyi and Oyedunmade (2004). The exhibited nematicidal properties may be due to the presence of bioactive chemical component including Saponin, flavonoid and tannin in the citrus peel extracts (Olabiyi *et al.*, 2008).

The significance of this study thus underpin the potency of citrus peel extracts as a viable alternative to synthetic nematicide in the control of nematode pests on susceptible crops. The Agro-chemical companies can

start extracting the bioactive components of the citrus peel as raw materials for manufacturing of botanical based nematicide as a guarantee for environmental safety and production of safe to eat food.

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