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EVALUATION OF SWEET ORANGE PEEL AQUEOUS EXTRACT (*Citrus sinensis*) AS ROOT –KNOT NEMATODE SUPPRESSANT

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ABSTRACT
The toxic effect of sweet orange peel (Citrus sinensis) was compared with that of carbofuran, a synthetic nematicide, for the suppression of soil and root population of root-knot nematode Meloidogyne incognita on tomato. Field and laboratory experiments were conducted in year 2002 and repeated in the same period in year 2003 at Ahmadu Bello University, College of Agriculture, Kabba, Kogi State, Nigeria. Sweet orange peel aqueous extract was applied at 0, 25, 50, 75 and 100% concentration, while carbofuran was applied at 0, 2500, 5000, 7,500 and 10,000ppm on the field and 0, 250, 500, 750 and 1000ppm respectively 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 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) compared with the control. The higher concentrations (7500 and 10,000ppm) of sweet orange peel aqueous extract were significantly more effective than lower concentrations of 2500 and 50,000ppm in suppressing nematode population in the soil and root as the performance is at par with carbofuran at that dosage level. The result indicates with respect to all the tested parameters that sweet orange peel is toxic to root-knot nematode and can be incorporated into its control system as a potential raw material for manufacturing organic based nematicide.

Key Words: Orange Peel, root-knot nematode; carbofuran (*furadan*), *Citrus sinensis*

INTRODUCTION

Tomato crop, grown both as a perennial and an annual 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, 1983). It is a source of vitamins A, C and D. This choice crop is affected by wide range of pests. Prominent among them is the plant parasitic nematode of which *Meloidogyne incognita* is identified as wide-: spread and very devastating on tomato crop.

The disease caused by this group of nematode is the root-knot disease and it's characterized by numerous pronounced swelling or galls on the roots 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 more rapid control is achieved by the use of chemicals known as nematicides. However, nematicides are expensive and can be hazardous both to the crop and the soil. All these problems have greatly limited the use of chemical nematicide by many farmers in Nigeria (Egunjobi and Onifade 1994). The search for alternative control measures for root-knot nematode has thus become imperative (Magbool *et a.,l* 1987). Alam *et al.,* (1980) and Verma (1976) have suggested the use of plant extracts. Plant extracts are cheap and are readily available compared with conventional nematicides, and are environmentally safe (Oyedunmade *et al.,* 1995; Zurren and Khan 1984; Egunjobi and Obayemi 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 the focus of research efforts because of their ability to produce environmentally less harmful, but efficacious chemical substance (Schmutterer 1990). This will greatly minimize the use of toxic synthetic chemicals. It is against this background that peel extracts of sweet orange (*Citrus sinensis*) was tested for its toxicity on root-knot nematode *M. incognita*, and to determine its

biocidal activity on root-knot nematode *M. incognita* since the toxicity of its component to other pests especially the field insects have been reported by researchers (Abolusoro, 2001; Olaifa, 1987; Helen *et al.*, 1972).

If the approach is successful, it will be of great benefit in developing a new control method and bringing in additional use for agricultural by product.

The study was therefore, conducted to (1) assess the effects 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 extracts on the soil and root population of root-knot nematode *M. incognita* and the root damage of tomato growing in the medium.

MATERIALS AND METHODS

The trial was conducted on a field at Ahmadu Bello University, College of Agriculture, Kabba, between the months of July and December 2002 and was repeated at the same period in the year 2003. Tomato (CV ROMA) seedlings were raised in sterilized nursery soil. The seedlings were transplanted to the permanent field on ridges 3 weeks after seedling emergence. The two factors were studied in rates for two seasons in Randomized Complete Block Design and each treatment consisted of one healthy seedling spaced 50cm x 90cm (intra-x inter row). Sweet orange peel aqueous extract was applied at 0, 25, 50, 75 and 100% concentration, while carbofuran was applied at 0, 2500, 5000, 7,500 and 10,000ppm on the field. Each plant stand was inoculated with 2000 eggs of *M. incognita* six weeks after planting (6 WAP). Fresh peel of sweet orange (*C. sinensis*) were collected, air dried and ground into fine powder. Weighed on balance and soaked in 1 litre of distilled water for 24hrs. The resultant filtrate was taken as the stock solution of strength 100% (1,000,000ppm). Several dilutions 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 stand at 6 weeks after planting (6 WAP), while the untreated control was denoted as 0%. Data were collected on number of nematode in 200g soil after harvesting by methods of Whitehead and Hemming, (1965). This is for determining the final population of nematode from a mean composite of four 200g sample of soil collected from around the root zone of uprooted individual plant. The population of juvenile *M. incognita* in 5g root was determined by the method described by Byrd *et al*., (1983). The root damage, otherwise known as gall index, was determined using a rating scale 0-5 (Taylor and Sasser, 1988). The test material (*Citrus* peel) was subjected to phytochemical screening so as to determine the presence of the bioactive chemical components.

The presence of Flavoniods, Tannins and Alkaloids were determined by the use of method described by Trease and Evan (1989). Saponin was tested by using the method described by (Trease and Evans 1978; and Sofowora 1982).

All data except on bioactive chemical components were subjected to analysis of variance and means separated by Duncan Multiple Range Test (DMRT).

Laboratory Experiment

Eggs of *Meloidogyne incognita* were extracted using sodium hypochlorite (Hussey and Baker, 1973) from galled roots obtained in a culture of root-knot nematode *M. incognita* from previous tomato (*Lycopersicon esculentum)* experiment. One hundred freshly extracted eggs were introduced into each of the fourty petri-dishes which were arranged in laboratory at room temperature of 28^0C. Twenty millimeters (20 ml) of each of the concentrations (100, 50, and 0%) of the aqueous extract of the citrus peel were added separately to eggs in the hatch; every treatment was replicated eight times. Observations on cumulative egg hatch were made every 24 hours for six consecutive days. This was done by counting the number of second stage juvenile which emerged from egg using stereomicroscope.

Juvenile mortality observation*:* Ten millimeters of carbofuran solutions at 0, 250, 500, 750 and 1000ppm concentrations and 0, 25, 50, 75 and 100% aqueous citrus extracts were introduced into each of the petri-dishes. Distilled water only served as control (0%). Standardized 1ml juvenile suspension containing 100 juveniles of *Meloidogyne incognita* was introduced into each of the transparent petri-dishes containing different concentrations of peel extract and carbofuran solution and the control. The contents of the petri-dishes were incubated at a temperature of 28 ± 2^0c. The petri-dishes were covered with glass to prevent evaporation. Each treatment was replicated five times in completely randomized design. Counts of dead juveniles were made initially after 6hrs and then 2 hours of setting up the experiment. Thereafter, the observations were made at 24 hours interval for a period of seven 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. This result shows that these parameters under study were significantly lower in the carbofuran treatments as compared with the citrus aqueous treatment. All the treatments reduced nematode population both in the soil and in the root at harvest and also root damage (gall index) was reduced in all the treatments as compared with the control. The level of reduction in soil and root population by both carbofuran and citrus peel treatment is directly proportional to the quantity of treatment applied. The effects of carbofuran and citrus peel treatments on root damage also followed the same trend as reduced galls which were significantly different from the control. On Table 2 is shown the comparative effect of carbofuran and citrus peel extract on egg-hatch inhibition of *Meloidogyne incognita*. The result shows that egg-hatch inhibition was significantly higher in carbofuran treatments as compared with citrus peel extract treatment and the control. The treatments inhibited egg-hatch but the level of inhibition was significantly higher in carbofuran treatments than the citrus peel extract treatments and the control. Few juvenile emerged at the lower level (25%) of citrus extract treatment which increased from average of 1.5% on day 1 to 9% at day 7 of the experiment white carbofuran treatments recorded an average of 1.5 juveniles hatch on day 2 of the experiment to average of 2% juvenile hatch on day 7 of the experiment. However, the (untreated) control recorded an average of 17.75 juveniles hatch on day 1 and 91.00% on day 7 of the experiment and it follows the same trend in the second trial of the experiment. (See Tables 2 and 3).

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

Treatment Concentration	Initial *M. incognita* Population (P1)		Final *M. incognita* Population on 200g soil (PF)		Nematode Multiplication rate (%) PF/P1 x 100		Number of Juvenile in 5g root		Gall index	
	2002 Trial	**2003 Trial**	**2002 Trial**	**2003 Trial**	**2002 Trial**	**2003 Trial**	**2002 Trial**	**2003 Trial**	**2002 Trial**	**2003 Trial**
Citrus 0 *sinensis*	2098	2111	2458.00i	2426.20h	117.1	1149	24.67e	26.67e	4.50c	4.25c
25%	2100	2112	969.25h	925.3f	46.11	43.81	17.33d	18.33d	3.20b	3.3ab
50%	2097	2101	877.21g	896.51g	41.81	466.7	13.67c	13.00c	2.88a	2.90ab
75%	2099	2103	812.50e	784.20e	38.89	37.29	12.00c	12.33c	2.97a	2.88a
100%	2112	2110	644.90c	648.21c	30.49	30.72	10.67c	10.67b	2.83a	2.83a
Carbofuran 0	2099	2110	2449.90i	2464.80h	129.50	116.78	27.00e	29.33e	2.50c	4.25c
2500 ppm	2112	2114	774.00f	780.22e	36.64	36..90	13.00c	16.00c	3.13b	3.38ab
5000 ppm	2097	2104	669.00d	660.0d	31.80	36.96	11.67c	12.00c	2.93a	2.88a
7500 ppm	2099	2097	565.71b	602.0b	26.95	28.10	8.00b	11.00b	2.97a	2.88a
10,000 ppm	2103	2100	466.50a	459.0a	22.18	21.85	5.67a	6.67a	2.8a	2.3a
SE	NS	NS	21.94	45.38	-	-	6.77	6.66	0.17	0.17

Values with the same letter (s) in the column do not differ significantly at P = 0.05 according to Duncan's Multiple Range Test.

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

		First Trial						
Treatment	**Concentration**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Citrus Sinensis	25%	1.50a	1.50a	7.00b	9.00b	9.00c	9.00c	9.00e
	50%	0a	0a	0a	0a	0a	0a	0a
	75%	0a	0a	0a	0a	0a	0a	0a
	100%	0a	0a	0a	0a	0a	0a	0a
Carbofuran	250ppm	0a	1.5a	1.50a	1.50a	2.00b	2.00b	2.00b
	500ppm	0a	0a	0a	0a	0a	0a	0a
	750ppm	0a	0a	0a	0a	0a	0a	0a
	1000ppm	0a	0a	0a	0a	0a	0a	0a
Control	0	17.75b	33.00b	38.00c	62.00cb	75.50d	91.00d	91.00d
SE		**1.032**	**2.116**	**1.652**	**1.229**	**1.442**	**0545**	**545**

Values 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 effects of carbofuran and *Citrus sinensis* peel extracts on egg hatch inhibition on *M. incognita*

		Second Trial						
Treatment	**Concentration**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Citrus Sinensis	25%	0a	1.37a	5.0a	7.2b	9.00c	8.5c	9.4
	50%	0a	0a	0a	0a	0a	0a	0a
	75%	0a	0a	0a	0a	0a	0a	0a
	100%	0a	0a	0a	0a	0a	0a	0a
Carbofuran	250ppm	0a	1.2a	1.3a	2.2a	2.2b	2.2b	2.2b
	500ppm	0a	0a	0a	0a	0a	0a	0a
	750ppm	0a	0a	0a	0a	0a	0a	0a
	1000ppm	0a	0a	0a	0a	0a	0a	0a
Control	0	16.92b	35b	41c	60b	79d	93d	3d
SE		**1.040**	**2.118**	**1.70**	**1.230**	**1.421**	**.508**	**.548**

Values followed by the same letter (s) in the same column are not significantly different at p=0.05 using Duncan's Multiple Range Test.

Table 4 Comparative effects of carbofuran and citrus peel extracts on mortality of *Meloidogyne incognita* Juveniles under laboratory condition.

		First trial								
Treatment	**Concentration**	**6hrs**	**12hrs (%)**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Citrus Sinensis										
	25%	0a	10.50b	71.00b	96.00b	100b	100b	100b	100b	100b
	50%	1.00b	19.00c	87.00c	100c	100b	100b	100b	100b	100b
	75%	10.00b	24.00d	100d	100c	100b	100b	100b	100b	100b
	100%	20.00c	40.50g	100d	100c	100b	100b	100b	100b	100b
Carbofuran	250ppm	8.00b	27.00e	100d	100c	100b	100b	100b	100b	100b
	500	16.00d	36.00f	100d	100c	100b	100b	100b	100b	100b
	750	25.00f	52.50h	100d	100c	100b	100b	100b	100b	100b
	1000	32.00g	60.00 i	100d	100c	100b	100b	100b	100b	100b
Control	0	0a	0a	0a	0a	0a	3.35a	6.00a	8.05a	10.0b
SE	0.757	1.641	1.256	0.200	0.00	0.143	0.612	.306	2.65	
		Second Trial								
Treatment	**Concentration (%)**	**6hrs**	**12hrs**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Citrus Seninsis	25%	0a	10.5b	10.5b	100b	100b	100b	100b	100b	100b
	50%	5.7b	19.01c	72.2b	100b	100b	100b	100b	100b	100b
	75%	16.00d	25.0d	81.51e	100b	100b	100b	100b	100b	100b
	100%	22.0f	41.21f	100e	100b	100b	100b	100b	100b	100b
Carbofuran	250ppm	8.5c	26.1d	100e	100b	100b	100b	100b	100b	100b
	500ppm	16.3d	37.0e	100e	100b	100b	100b	100b	100b	100b
	750ppm	24..9g	54.0g	100e	100b	100b	100b	100b	100b	100b
	1000ppm	34h	62.0h	100e	100b	100b	100b	100b	100b	100b
Control	0	0a	0a	0a	0a	0a	0a	0a	0a	0a

Values followed by the same letter (s) in the same column are not significantly different at p =0.05 using Duncan's Multiple Range Test. (DMRT)

The effect of carbofuran and citrus peel extract at different concentrations on juvenile mortality of *M incognita* is on Table 4. The result shows that both carbofuran and citrus peel extract are effective in causing mortality in *M. incognita* juvenile. Mortality was observed at all the concentration levels of carbofuran and citrus peel treatment from 6 hours after treatment application. A 100% mortality was observed at all the levels of carbofuran 24 hours 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.

The results of the bioactive chemical components showed that citrus peel contains 9.78% sapomins, 6.22% tannins, no alkaloid and 44 mm^2 of flavonoids. This chemical components exercise some degree of toxicity to nematode.

DISCUSSION

The citrus peel extract and carbofuran significantly reduced the soil root population of *Meloidogyne incognita*. Root damage was equally reduced in both treatments in proportion to the levels of application and significantly different from the control treatments. This observation agrees with those of earlier researchers (Viala *et al.*, 1998; Sellami and Moufarrah, 1994). Abidel *et al.*, (1995) reported the effectiveness of various plant extract used in their various experiments in suppressing both 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 nemato-toxic effects of the peel extract can be attributed to the presence of nematicidal chemical components that are seriously injurious to *Meloidogyne incognita* eggs and the second stage juveniles. The findings from the study corroborate those of earlier researchers like Pandy (1990), Kumar and Singh (1972) and Mani and Al Hinai (1998) who reported the toxicity of various plant extracts used in their experiments.

Saponins, flavonoids, and tannins were present in the peel and are probably responsible for the toxicity of the peel extracts to *M. incognita*. This observation agrees with Daveral (1972), Alam *et al* (1978), and Barry *et al* (1986) 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 effects of citrus extracts on plant parasitic nematode have been reported by many workers including Oyedunmade (2004), Oyedunmade (2001) and Olabiyi (2004). The exhibited nematicidal properties may be due to the presence of bioactive chemical components including Saponin, flavonoid and tannin in the citrus peel extracts (Olabiyi *et al* 2008).

The significance of this study thus underscores the potency of citrus peel extracts as a viable alternative to harmful synthetic nematicide for the control of nematode pest 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.

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