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Effect of Essential Oils from Five *Ocimum* sp. on the Pathogenicity of *Pratylenchus brachyurus* (Godfrey) in Tomato

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Abstract: The effect of the essences from hydrodistillation of five *Ocimum* sp. on *Pratylenchus brachyurus* was compared with carbofuran in *in vitro* and greenhouse trials. Nematotoxic concentrations of oil were linearly proportional, but varied from one plant to another. At 25-100 μg mL^{-1}, the oils of *O. gratissimum* and *O. basillicum* completely inhibited egg hatching and larval survival of nematodes after 24 h. 60.4% of nematode larvae were ruptured following a 22 h exposure to a binary combination of *O. gratissimum* and *O. basillicum* oils at 100 μg mL^{-1}. The undiluted broth obtained after the isolation of *O. gratissimum* oil induced 66.1% larval mortality and inhibited egg hatching by 62.8%. The essential oil of *Ocimum forskolei* exhibited a weaker toxicity; inactivating most of the nematodes at 100 μg mL^{-1}. Essential oils of *O. canum* and *O tenuiflorum* had no effect on the nematode even at 100 μg mL^{-1}. The nematicidally potent essential oils of *O. gratissimum* and *O. basillicum* were not phytotoxic to tomato plants at 6.25-100 μg mL^{-1}. A significant ($p<0.05$) improvement in growth of potted tomato plants in the greenhouse was associated with the essential oils of *O. gratissimum* and *O. basillicum*. Plants receiving these treatments were taller and greener, developed fewer root galls and produced more fruits than plants treated with the essential oil of *O. forskolei*. Amending soil with a binary combination of *O. gratissimum* and *O. basillicum* oils at 100 μg mL^{-1} was significantly more efficacious than either oil at 100 μg mL^{-1} and compared with carbofuran treatment at 100 μg mL^{-1}. The observed differences in the nematicidal potential of the control plants are natural in terms of the overall chemical composition of the essential oils.

Key words: *Ocimum*, essential oil, *pratylenchus brachyurus*, hatching inhibition, juvenile mortality, tomato, galling index

INTRODUCTION

The damage caused by the root-lesion nematodes (*Pratylenchus* sp.) to crops is a major impediment to food production in the semi-arid regions. In Nigeria, *Pratylenchus brachyurus, P. coffeae, P. zeae* and *Psafaensis have* been found to induce severe losses in crop yields, ranking high amongst the major destructive pests encountered in the sub-region (Adesyian *et al*., 1990; Moghal *et al*., 1993). Among these nematode *P. brachyurus* sp. has been a serious constraint to food production where it occurs; infected plants are characterised by chlorosis, stray foliage, necrosis of the feeder root and loss in crop yield or death of the plant. The nematode causes lesions by feeding on and killing root cells, thereby impairing the uptake of minerals and water from the soil.

Of the various methods available for controlling root-lesion nematodes, the use of synthetic nematicides has been the most effective (Moghal *et al*., 1993). However, inappropriate uses of synthetic nematicides have led to pest resistance, high toxic residues in foods, ecological imbalance and constituted danger to farmers (Thomason 1987; Shashikant and Sahai 1989; Lingk, 1992). Many of the chemical nematicides are expensive and have been banned in several countries due to their damaging effects on human health. There is thus a strong need to explore local sources for cheaper nematicides, which are also environmentally friendly. The practical use of natural compounds as control agents is receiving increased attention and this is partly due to their non-toxicity to humans and/or non-target, their sustainability and biodegradability (Grainge and Ahmed 1988).

Several studies have documented the nematicidal potentials of essential oils from plants, especially in the families Labiatae, Lamiaceae, Meliaceae, Rutaceae, Rubiaceae, Umbeliferae, Asteraceae, Sterculiaceae and Ceasalpinaceae. Essential oils are a mixture of monoterpenes, sesquiterpenes and other non-terpenoid compounds (Dormans and Deans, 2000; Al- Burtamani *et al*., 2005; Fatope *et al*., 2006). Most of the researches on the nematotoxic efficacy of essential oils and their

components have been directed against the root-knot nematodes (Chatterjee *et al*., 1982; Sangwan *et al*., 1985; Soxena *et al*., 1987; Babul and Sukul, 1990; Pandy, 1990; Sangwan *et al*., 1990; Gosh and Sukul, 1992; Leela *et al*., 1992; Walker and Melin, 1996; Oka *et al*., 2000; Pandey *et al*., 2000; Oka, 2001; Al- Banna *et al*., 2003) and to date, relatively little is known about the inhibitory effects of the essential oils of *Ocimum basilicum, O. canum, O. forskaolei, O. gratissimum* and *O. tenuiflorum* on root-lesion nematodes. What is well known, however, is that different parts of these plants have been used in local medicinal practices to combat a wide range of disease conditions and they possess strong pesticidal properties.

The juice express from the leaves of *Ocimum* is used as a remedy for headache, arthritis as well as skin and gynaecological disorders in the Northern part of Nigeria. The leaves of *Ocimum* are used as anti-helmintics (Orifadiya *et al*., 2001; Bais *et al*., 2002) and to treat ulcer, rheumatism, diabetics, diarrhoea, dysentery, malaria and high blood pressure (Nagassoun *et al*., 2003; Opalhenova, 2003; Passeual-Villalobos, 2003; Nakamura *et al*., 2004). There are reports that extracts of *Ocimum* sp. are effective against HIV-1 and HIV-2 infections (Asha *et al*., 2001). Essential oils from *Ocimum* are reputed for their insecticidal and antimicrobial properties (Pessoa *et al*., 2002; Offian and Chikwendu, 2003; Harris, 2001; Grover *et al*., 2002; Obot and Alugi, 2002; Dharmani *et al*., 2004; Ayasi and Nyadedzor, 2003).

The present investigation was designed to provide information on the effectiveness of the essential oils of five *Ocimum* sp. in controlling *Pratylenchus brachyurus*. The relative nematicidal activity of the essential oils was estimated from their lethality to eggs and larvae; effect on plant growth and on the population of nematodes in root and soil.

MATERIALS AND METHODS

Plant material and essential oil: The aerial parts of five Lamiaceous plants including *Ocimum basillicum, O. canum, O. forskolei, O. gratissimum* and *O. tenuiflorum,* were collected from different parts of South-western Nigeria between March and June 2005 and identified by Dr. Shahina A. Ghazanfar (Taxonomist, Herbarium, Royal Botanic Garden, Kew, U.K.). Voucher specimens have been deposited in the herbarium of the Department of Forestry and Wood Technology, Federal University of Technology, Akure, Nigeria. Fresh twigs (500g) of each plant were cut into small pieces and hydrodistilled using a Clevenger-type apparatus for 3 h. The steam distillate was dried over anhydrous sodium sulphate (Fatope *et al*., 2006).

One g of the essential oil was diluted with 10 mL of 0.01% Tween-20 to obtain a 100 μg mL^{-1} stock solution. Carbofuran (carbarmate nematicide) was similarly diluted with distilled water. Serial dilutions of each solution gave rise to a concentration gradient of 50, 25, 12.5, 6.25 μg mL^{-1}, respectively. The aqueous residue (broth) recovered after the isolation of *H. tuberculatum* oil was also serially diluted with sterile distilled water. Equal volumes of 100 μg mL^{-1} *O. gratissimum* and 100 μg mL^{-1} *O. basillicum* oils were mixed together to obtain a binary combination of each oil at 50 μg mL^{-1}.

***In vitro* nematicidal assay:** Eggs of *Pratylenchus brachyurus* used were extracted from the infected roots of tomato (*Lycopersicon esculentum*, cv. local) using the sodium hypochlorite technique (Hussey and Barker, 1973). Some of the eggs were allowed to hatch to the second stage larvae (J_2) and used for toxicity tests within 4 days.

Approximately 110 active second stage larvae or eggs of *Pratylenchus brachyurus* in 500 μL of distilled water were separately mixed with 5 mL of test oil or broth or carbofuran solution in a 6 cm-diameter Petri dish. Dishes containing 500 μL of nematode suspension and 5 mL of distilled water or 5 mL of 0.01% Tween-20 were used as a blank control. Each treatment was replicated five times and incubated at 27°C. Percentage larval mortality and egg hatching inhibition were determined by counting under a microscope after 24 h and 7 days, respectively using the expression: 100-(100SE/SC); where, SE = number of eggs that hatched or number of larvae that survived in essential oil and SC=number of eggs that hatched or number of larvae that survived in control (Onifade, 2006).

Greenhouse experiments: Nematode-free sandy-loam soil in each of 2 litre-capacity plastic pots was infested with 50 mL of the *Pratylenchus brachyurus* eggs suspension. There were 110 eggs per mL of suspension. Five days after inoculation, soil in each pot was drenched with 100 mL of the respective test solutions and the controls. Untreated but inoculated control pots were similarly treated. One actively growing but highly nematode-susceptible seedling of tomato (*Lycopersicon esculentum* cv. West coast TM782C) was transplanted into each pot when three weeks old. Each treatment was replicated five times and randomly arranged on greenhouse benches. During the growing period, all experimental plants were watered daily, kept weed-free and their heights (cm) were measured on a weekly basis until maturity.

Twelve weeks after transplanting, the fruits were bulk-harvested and weighed (g $^{-1}$plant), after which the roots were removed from the potted soil and rated for

Table 1:Mean Percentage hatching inhibition of *P. brachyurus* eggs inessential oils

	% Hatching inhibition (± S.E)				
	Oil concentration (μg mL^{-1})				
Plants	6.25	12.5	25	50	100
O. canum	0±0.0d*	0±0.0c	0±0.0c	0±0.0c	0±0.0c
O. gratissimum	53.8±0.4b	98.3±0.5a	100±0.0a	100±0.0a	100±0.0a
O. forskolei	0±0.0d	6.3±0.8b	10.6±0.9b	32.7±0.8b	38.3±0.6b
O. basillicum	22.5±0.2c	97.9±0.4a	100±0.0a	100±0.0a	100±0.0a
O. tenuiflorum	0±0.0d	0±0.0c	0±0.0c	0±0.0c	0±0.0c
Mixture of *Ob/Og*	78.1±0.9a	92.4±0.1a	100±0.0a	100±0.0a	100±0.0a
Carbofuran	81.8±1.3a	100±0.0a	100±0.0a	100±0.0a	100±0.0a

*Values with the same letter within the same treatment level are not significantly different ($p<0.05$) using multiple range test. *Ob: Ocimum basillicum, Og: Ocimum gratissimum*

Table 2: Mean percentage mortality of *P. brachyurus* larvae in essential oils after a 24 h

	% Juvenile mortality (+SE)*				
	Oil concentration (μg mL^{-1})				
Plants	6.25	12.5	25	50	100
O. canum	0±0.0d	0±0.0c	0±0.0c	0±0.0c	0±0.0c
O. gratissimum	28.5±0.9c	90.3±0.3a	100±0.0a	100±0.0a	100±0.0a
O. forskaoli	0±0.0d	16.1±0.8b	30.9±0.6b	43.9±0.5b	58.5±0.9b
O. basillicum	35.9±0.6c	87.2±1.0a	98.2±0.3a	100±0.0a	100±0.0a
O. tenuiflorum	0±0.0d	0±0.0c	0±0.0c	0±0.0c	0±0.0c
Mixture of *Ob/Og*	51.7±0.8b	90.5±0.4a	100±0.0a	100±0.0a	100±0.0a
Carbofuran	85.5±0.9a	100±0.0a	100±0.0a	100±0.0a	100±0.0a

*Values within the same treatment level followed by the same letter are not significantly different ($p<0.05$) using the multiple range test. *Ob: Ocimum basillicum, Og: Ocimum gratissimum*

galling on a 0-5 scale (Taylor and Sasser, 1978). Nematode eggs and larvae were recovered from the galled roots using a 0.5% sodium hypochlorite solution, while the soil in each pot was also extracted for nematode counts using the modified Bearmann funnel technique (Whitehead and Hemming, 1965). Final nematode numbers in soil and tomato roots were then recorded.

Phytotoxic screening of oils: Thirty healthy seeds of tomato (cv. West coast TM782C) were soaked in 5 mL of 100 μg mL^{-1} *Ocimum* oils in sterile Petri dishes for 48 h. Water-soaked seeds were kept as control. Thereafter, the treated seeds were planted in nematode-free potted soil (5 seeds per pot) and maintained on greenhouse benches. Observations were made on emergence of the plumule as well as on height of the test plants.

Statistical analysis: The experiments were repeated twice and each treatment was replicated five times. Pooled data were subjected to two-way analysis of variance using a computer-aided SPSS statistical procedure and mean comparisons were based on Duncan multiple range test (Duncan, 1955). The direct relationship between *P. brachyurus* populations (in soil and roots) and tomato growth indices were calculated using correlation coefficient analysis.

RESULTS

The results of *in vitro* nematicidal assays in Table 1 and 2 show that the essential oils of *Ocimum gratissimum, O. forskolei* and *O. basillicum* were directly nematotoxic to *P. brachyurus*. The susceptibility of nematodes to essential oils at the same concentration varied between plants, although nematicidal efficacy increased with increased concentration of the essential oils. When used singly or combined, *O. gratissimum* and *O. basillicum* oils were most efficacious and compared favourably with carbofuran in suppressing nematode eggs hatching and killing larvae at 25-100 μg mL^{-1}. Thirty-five *P. brachyurus* larvae were ruptured on exposure to a binary combination of *O. gratissimum* and *O. basillicum* oils at 100 μg mL^{-1} for 22 h. Aqueous residue or the broth obtained after steam distillation of *O. gratissimum* oil also inhibited nematode egg hatching by 62.8% and was lethal to 66.1% of the larvae. The broth induced Lethal Concentration (LC_{50}) values of 87 μg mL^{-1} and 90.4 μg mL^{-1} on the nematode's larvae and eggs respectively using the Finney computer program. The essential oil of *O. forskolei* exhibited weak toxicity to the nematodes. The oil of *O. canum* and *O tenuiflorum* were not lethal to the nematode larvae and had no effect on egg hatching even at 100 μg mL^{-1}. The oils from these plants were therefore not included in the greenhouse experiments.

Table 3: Effects of essential oils on heights of tomato plants grown in *Pratylenchus*-infested soil

	%Increase in plant height				
	Oil concentration (μg mL^{-1})				
Plants	6.25	12.5	25	50	100
O. gratissimum	0.5±0.0c*	43.4±0.9b	68.3±1.1a	71.2±0.6b	74.7±0.7b
O. basillicum	0±0.0c	41.1±0.4b	66.1±0.6a	66.9±0.2b	70.4±0.3b
O. forskolei	0±0.0c	23.8±1.9c	47.8±1.4b	50.1±0.6c	58.3±0.8c
Mixture of *Ob/Og*	17.2±2.1b	60.0±0.6a	72.2±0.8a	88.2±0.1	90.1±0.3a
Carbofuran	56.2±0.9a	65.7±1.2a	78.9±1.3a	88.4±0.5a	91.2±0.6a

*Each value is a mean of five replicates±standard error of the mean. *Means followed by the same letter within the same treatment level are not significantly different($p<0.05$) using multiple range test

Table 4: Effect of essential oils on fruit yields of tomato plants grown in *P. brachyurus* -infested soil

	%Increase in fruit yield + S.E				
	Oil conc. (μg mL^{-1})				
Plants	6.25	12.5	25	50	100
O. gratissimum	2.1±3.5c	28.7±1.8c	46.2±3.1c	58.3±1.1b	70.1±1.3b
O. basillicum	0±0.0d	20.8±1.1c	33.6±2.7c	49.1±2.4b	58.2±1.5c
O. forskolei	0±0.0d	17.7±3.2d	28.1±4.2d	40.7±3.3c	51.3±2.1c
Mixture of *Ob/Og*	21.6±4.4b	43.4±1.5b	61.8±2.2b	80.3±1.8a	91.7±1.0a
Carbofuran	51.4±2.1a	65.7±1.9a	79.1±1.3a	100±0.0a	100±0.0a

*Each value is a mean of five replicates±standard error of the mean. Means followed by the same letter within the same treatment level are not significantly different($p<0.05$) using multiple range test

Table 5: Effects of essential oils on tomato root gall ratings

	Mean root gall index				
	Oil concentration (μg mL^{-1})				
Plants	6.25	12.5	25	50	100
O. gratissimum	4.2a*	2.0b	2.0bc	2.0c	1.0c
O. basillicum	4.2a	2.2b	2.2bc	2.0c	2.0b
O. forskolei	4.4a	4.1a	3.0b	2.8b	2.0b
Mixture of *Ob/Og*	3.2b	2.0b	1.2c	1.0d	1.0c
Carbofuran	1.2c	1.0c	0d	0e	0d
Untreated control	4.4a	4.4a	4.4a	4.4a	4.4a

*Means within the same treatment level followed by the same letter(s) do not differ significantly ($p<0.05$) using multiple range test

Nematode-susceptible tomato plants (*L. esculentum* cv. West coast TM782C) were cultivated in the greenhouse. Potted plants that received carbofuran and mixtures of *O. gratissimum* and *O. basillicum* oils at 25-100 μg mL^{-1} produced fewer galls and were generally taller with better fruit yields than those in untreated but nematode-infested plots (Table 3-5). Plants cultivated on nematode-infested soil and amended with 6.25 and 12.5μg mL^{-1} oils respectively, were stunted, chlorotic with very low fruit yields and showed mid-day wilting when six weeks old. Their roots developed heavy galls with mean gall index of 4.4 (Table 5). The lowest gall rating of 1.0 was recorded on the roots of plants treated with a combination of *O. gratissimum* and *O. basillicum* oils at 50 and 100 μg mL^{-1} respectively. The gall rating value was not significantly ($p<0.05$) different from that of plants treated with carbofuran at 6.25 and 12.5 μg mL^{-1}. The root gall index was negatively correlated with maximum plant height ($r = -0.84$), but positively correlated with nematode counts in tomato roots ($r = +0.80$) and in soil ($r = +0.91$).

The highest populations of *P. brachyurus* in the soil and within plant roots, were observed in *O. forskolei* oil-treated and the untreated nematode-infested plots, the least was in the pots treated with carbofuran and mixtures of *O. gratissimum* and *O. basillicum* oils at 25, 50, or 100 μg mL^{-1} (Table 6 and 7). The essential oils of *O. gratissimum* and *O. basillicum* and their mixtures compared well with carbofuran in suppressing nematode populations in the root. The effect of carbofuran on root nematode population at 50-100 μg mL^{-1} was not significantly different ($p<0.5$) from that of combined *O. gratissimum* and *O. basillicum* oils, also at 50-100 μg mL^{-1} (Table 6). Nematode populations in the roots were usually 2-3 times as numerous as those recovered from soil.

DISCUSSION

The essential oils of *Ocimum gratissimum* and *O. basillicum* have been found to be as effective as carbofuran (a synthetic nematicide) in inhibiting the

Table 6: Mean percentage reduction of *P. brachyurus* numbers in roots of tomato grown in soil treated with essential oils

	% Reduction (±S.E)*				
	Oil concentration (μg mL^{-1})				
Plants	6.25	12.5	25	50	100
O. gratissimum	22.3±0.9c	60.2±1.2b	73.9±0.4b	84.4±0.7a	90.4±0.3a
O. basillicum	22.1±2.5c	57.3±0.3b	76.4±0.7b	80.5±0.9a	89.1±0.5a
O. forskolei	3.6±1.4d	21.3±2.7c	39.5+1.5c	56.8±3.3b	59.7±1.7b
Mixture of *Ob/Og*	43.8±0.8	80.5±0.6	91.0±0.2	92.9±0.2	100±0.0a
Carbofuran	85.6±0.5a	95.4±0.6a	100±0.0a	100±0.0a	100±0.0a

*Values with the same letters within the same treatment level are not significantly different at 5% using multiple range test

Table 7: .Mean percentage reduction of *P. brachyurus* numbers in soil treated with essential oils

	% Decrease in soil nematode numbers (±S.E)*				
	Oil concentration (μg mL^{-1})				
Plants	6.25	12.5	25	50	100
O. gratissimum	22.6±0.9c	54.9±0.5b	72.8±0.9b	84.9±0.4	88.1±0.2
O. basillicum	21.9±1.3c	51.8±1.8b	74.3±0.5b	85.3±0.7a	87.7±0.6a
O. forskolei	15.5±2.1c	30.4±3.5c	43.9±2.7c	55.8±1.6b	57.3±1.9b
Mixture of *Ob/Og*	55.1±1.2b	75±0.2ab	90.4±0.3a	95.9±0.7a	100±0.0a
Carbofuran	84.3±0.2a	96.2±0.6a	100±0.0a	100±0.0a	100±0.0a

*Mean values with the same letter within the same treatment level are not Significantly different ($p<0.05$) using multiple range test

survival of *Pratylenchus brachyurus in vitro* and under greenhouse conditions. A binary combination of these oils was more effective in controlling the nematodes indicating a synergistic effect. For instance, at 100 μg mL^{-1} the mixed oil ruptured 60.4% nematode larvae following a 22-h exposure. It has been suggested that essential oils could interfere with the physiological environment to the detriment of the nematode (Renninger *et al*., 1958) or disrupt or change the permeability of the cell membrane of the nematodes (Oka *et al*., 2000).

The essential oils of *O. gratissimum* and *O. basillicum* significantly ($p<0.05$) reduced the populations of *P. brachyurus* in the soil and root when used as soil amendments. This suggests that the oils could have larvicidal activity or could inhibit the hatching of eggs in pot cultures (Walker and Melin, 1996; Oka, 2001; Miller, 1979). In this investigation, strong positive correlations occurred between root gall index and nematode numbers and suppression in populations of *P. brachyurus* in the root. One possible explanation for this is that the higher the nematode population in the root, the greater the damage done to the plant. Although a reduction in the severity of root-knot disease was observed after treatment with *O. gratissimum* and *O. basillicum* oils, a single treatment with one oil is not enough to effectively control the disease. The amount of infection was strongly reduced whenever combined treatments between *O. gratissimum* and *O. basillicum* oils were used, even at a concentration as low as 12.5 μg mL^{-1}. The observation that aqueous residue (or broth) left after the isolation of the essential oils showed some level of toxicity against the *P. brachyurus* suggests that water soluble components of the oils may play some role in their nematicidal properties.

CONCLUSION

Conclusively, the strong nematicidal efficacy of the essential oils of *O. gratissimum* and *O. basillicum* support the hope that the oils can effectively control root-knot nematode disease on agricultural crops. Further attempts are being made to elucidate the specific nematicidal principles inherent in the essential oils and the efficacy of the test samples under field condition.

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