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Effect of bromination and oxidation on the nematicidal potential of orange peel oil using *Pratylenchus penetrans* infecting maize

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Abstract

Study was conducted to investigate the effect of oxidation and bromination of fresh and decomposed orange peels on the nematicidal potential of orange peel oil against *Pratylenchus penetrans*. Results showed that the oxidised/brominated orange peel oils were significantly effective at ($p \leq 0.05$) than ordinary orange peel oils and compared with the standard mocap. The decomposed orange peel oil was effective than the fresh peel oil and showed 51.42% mortality as compared to 39.38% after 24 hrs. Plants received brominated/oxidised oils were taller with better yields than untreated and nematode infested plots in the field. The GC/MS result indicated that the major constituents of the orange peel oils were limonene, octanal and citroneloll.

Keywords: Bromination, oxidation, nematicidal potential, orange peel oil, *Pratylenchus penetrans*, maize

Globally, root-lesion nematodes are the most common pests of corn and several species in this group caused significant damage (Norton, 1983; Norton, 1984; Windham, 1998). Significant reduction in maize yield has been associated with *Pratylenchus* spp., infection. McDonald & Nicol (2005) reported poor growth and yield reduction in maize fields to *Pratylenchus brachyurus*, *P. zeae* and *P. penetrans*. *P. brachyurus* responsible for a 28.5% yield reduction in Nigeria. This reduction was correlated with a 50% increase in nematode density (Egunjobi & Larinde, 1975). *Pratylenchus* spp., caused root lesion through feeding on the root cells and thus impairing the uptake of nutrients from the soil. Infected plants were characterized by chlorosis, root necrosis, reduced yield and finally death of the plants. *Pratylenchus penetrans* are wide spread in maize growing areas of Nigeria. A decrease in maize yield, as well as delay in fruit ripening when nematode populations reached 230-590 nematodes per gram of root. Fungal and bacterial root pathogens in maize can be enhanced by the presence of *P. penetrans* (Shurtleff & Averre, 2000). Florini & Loria (1990) stated the suitability of maize as host to *Pratylenchus penetrans*, while McDonald & van den Berg (1993), also substantiated the reproducibility of *P. penetrans* on maize. Greenhouse studies have shown that *P. penetrans* can cause severe damage in corn (Paul & Dorrance, 2011). Several methods of control have been put in place to reduce the population of plant parasitic nematodes on maize, some of which include the use of synthetic nematicides. Synthetic compounds have effectively controlled many nematode species but their extensive use led to serious environmental problems. Many cases of lethal and sub lethal pesticide poisoning of humans have occurred. This upsurge in prevalence of side effects of many synthetic nematicides has encouraged scientists on the need for stimulated intensive research on plant based nematicides. Investigations were therefore carried out on the potential of orange peel an agricultural waste for its possible nematode population reduction in agricultural fields. *Citrus sinensis* is a fruit that is vastly rich in vitamins and minerals (Nicolosi *et al*., 2000). Grated orange peel is used as flavouring and essential oil from the peel is used commercially in soft drinks and candy flavours (Morton, 1987). Excessive contact with the volatile oils in the peel can result in dermatitis, while peel large quantity of oranges have rashes and blisters in between fingers (Morton, 1987). Orange peel oil was used in

aromatherapy to calm anxieties and relieved tension. It was also used to treat bronchitis (Hanson, 2003). Essential oils of different plants have been found to possess nematicidal activity (Chitwood, 2002). Orange oils used extensively in the treatment of colds, flu and to eliminate toxins from the body. It has been indicated in biological pest control and has pronounced safe for use in kitchens as esoteric oils.

The objective of the present research was to evaluate the effect of bromination and oxidation of orange peel oil on the population of *Pratylenchus* spp., infecting maize in the field and compare the nematicidal activity of the derivatised oils with a standard nematicide mocap.

Materials and Methods

Chemicals: Solvents were obtained from chemical store metropolis in Ilorin, Nigeria and diluted before use. Silica gel 60 $_{F}$254 for Thin Layer Chromatography (TLC) and vanillin spray reagent were obtained from the Chemistry Department, University of Ilorin, Ilorin, Nigeria.

Extraction of orange oil from fresh peels: Fresh orange peels (1kg) was cut into small pieces to facilitate the extraction of oil from the peels using steam distillation process as described by Vogel (1989). Dichloromethane (DCM) was used to extract the oil from the resulting steam distillate and dried using anhydrous magnesium sulphate (anh. $MgSO_4$). The solution was filtered and concentrated to 715 mg fresh orange peel oil (FOPO).

Extraction of orange oil from decomposed orange peels: Fresh orange peels (1 kg) was allowed to decompose for a week and mash extracted with dichloromethane. The organic layer was separated from the aqueous layer and solution dried over anhydrous magnesium sulphate. The extracted oil after concentration weighed 803 mg and coded (DOPO).

Oxidation of extracted oil: Fresh and decomposed concentrated orange peel oil (100 mg) was taken into a 50 ml flask separately and after that freshly prepared 1M $KMnO_4$ solution (10 ml) added. The solution turned brown and became deeper with the addition of a further 5 ml $KMnO_4$ .The oxidation product was extracted with dichloromethane, washed several times with water and finally dried over anh. $MgSO_4$ after filtration concentrated in vacuum. The products obtained were coded FOPO/Ox and DOPO/Ox.

Bromination of extract: Fresh and decomposed concentrated orange peel oil (100 mg) was used for bromination. The oil was first dissolved in a little amount of dichloromethane in a 50 ml flask and then bromine water added. Bromine water was added drop wise until a brown colour of bromine persisted. The bromination product was extracted from the solution by adding more dichloromethane. The bromide derivatives were coded FOPO/Br and DOPO/Br.

Spectroscopic measurement: Infra-red (IR) spectra of chromatographic fractions were recorded on SHIMADZU 8400s FTIR (Fourier Transform) spectrophotometer, while the GC/MS was taken on a Gas Chromatography-Mass Spectroscopy, system; GCMS-QP 2010 PLUS (Shimadzu, Japan) interfaced with a finigan MAT ion trap detector ion source Temp., RTX5MS column packed with 100% dimethylpolysiloxane was used with the following settings; 200 ^{o}C, interfaced Temp., 250 ^{o}C solvent cut time; 2.50 min; relative detector mode, ACQ mode; Scan; start time-end time; 3-46 min, event time, 0.50 sec and scan speed 1428. Identification of the volatile component was carried out using the peak enrichment technique of reference compounds and as final confirmation of the peak identification by GC-MS; their spectral were compared with those of NIST library mass spectra.

Laboratory experiment: *Pratylenchus penetrans* eggs were extracted from infected roots of tomato *Lycopersicon esculentum* using the method of Hussey & Barker (1973). Some of

the eggs were incubated at 27 °C and hatched out the second stage juveniles used in the mortality test. Approximately 100 eggs and nematode juveniles were used in each Petri dish for the toxicity tests. The experiment was a factorial design conducted in randomized complete design (RCD), comprising seven treatments at four levels and replicated three times *in vitro* experiment. Orange peel oils were applied at 15 mg/ml, 25 mg/ml and 35 mg/ml. A non-ionic surfactant emulsifier (1 ml) was added to achieve total solubility and to provide homogeneous solution of the orange peel oils. Distilled water served as control (0 mg/ml), while mocap a synthetic nematicide was used as a standard check.

Field experiments: Experiments were conducted during the 2012-13 growing season at the University of Ilorin Teaching and Research Farm (lat. 8^0, 29^1 NE, long. 4^0, 40^1 E of the Greenwich Meridian) in the southern guinea savannah ecological zone. Maize plots consisted of five 20 m long rows spaced 1 m apart with 30 cm plant spacing within the rows. Each plot was inoculated with tomato roots infected with *Pratylenchus penetrans*. Initial nematode population was taken before and after inoculation. Four seeds were sown per hole and this was thinned down to two plant stand after two weeks of germination. The experiment was conducted in randomized complete block design (RCBD) involving seven treatments at four levels and each replicated three times. This showed total of 84 plots for each of the experiments. Orange peel oils were applied at 40, 50 and 60 mg/ml. Mocap a synthetic nematicide was used as a standard check and applied in the solid form at 0.5, 1.0 and 1.5 a.i/ha.

Data were taken from the field on plant height. Days to 50% tasseling were recorded. Fruit weight per plant was taken after harvest while nematode population per 250 g of soil sample and nematode population in 15 g root sample recorded after the experiment in the laboratory. Counting of nematode populations in root and soil was done under the stereomicroscope at x50.

Statistical analysis: All data collected were subjected to analysis of variance (ANOVA) and significant means separated with the Duncan's multiple range tests ($P \leq 0.05$).

Results

Spectroscopic results: The major IR absorption peaks exhibited by the orange peel oils were: 3650; 3566; 3241; 2924; 2852; 1734; 1716; 1647 and 1458. The broad band in the range of 3624-3541cm^{-1} was a characteristic of OH stretching vibration. The absorption band at 2924 cm^{-1} emphasizes the presence of aliphatic CH stretching. The alkene moiety was supported by the peak at 1458 and 1647 cm^{-1}. As a result of oxidation, ether C-O absorption is substantiated by the peak at 1261 cm^{-1}. C-Br stretch 569 cm^{-1} indicated the establishing bromination occurred. The GC/MS result of the fresh peel oil showed twelve peaks indicating the presence of at least twelve compounds.

Limonene, citronellol and octanal were the major constituents. GCMS results of other oils revealed more compounds different from obtained in the fresh peel oil, indicating the formation of new products in the decomposed oxidised/brominated decomposed and fresh oils. However, limonene remains the main constituents by the GC/MS result. Furthermore, TLC chromatogram of the fresh peel oil displayed fewer compounds, while those of the decomposed peels and oxidised/brominated fresh and decomposed peel oils exhibited other compounds. Fresh orange peel oil GC/MS analysis showed the presence of twenty eight (28) constituents from which sixteen (16) were identified (Table 1).

Nematicidal test results: The tested oils were effective in suppressing egg hatch and inducing juveniles mortality. However, all treatments significantly ($p \leq 0.05$) inhibited egg hatch (Table 2 and 3).

Table 1. Chemical identified in fresh orange peel oil through GC/MS analysis.

GC Peak No.	Compound	Rt (min)	Peak area (%)
1	Octanol	2.09	2.80
2	α-pinene	4.73	3.11
3	Sabinene	5.21	1.12
4	D-limonene	6.03	36.05
5	Octanal	6.13	12.15
6	α-terpeneol	6.26	3.08
7	Geranial	6.40	2.20
8	Citronellol	7.32	21.11
9	α-humulene	7.58	3.12
10	β-elemene	8.10	Trace
11	β-selinene	8.39	Trace
12	Myrtenal	9.02	3.10
13	Myrcene	9.41	4.12
14	α-pinene isomer	10.33	1.13
15	Neral	11.21	3.12
16	Llinalool	12.11	4.03

Table 2. Effect of treatment and level of application of orange peel oils on juvenile mortality of *Pratylenchus penetrans*.

Treatments	Exposure time (hrs)				
	2	4	6	8	24
FOGPO	5.33^{d}	9.24^{d}	13.51^{d}	20.22^{d}	39.38^{d}
FOGPO/Ox	10.09^{b}	14.78^{b}	26.17^{b}	35.64^{b}	62.79^{b}
FOGPO/Br	10.67^{b}	15.16^{b}	26.30^{b}	36.12^{b}	63.10^{b}
DOGPO	8.21^{c}	11.09^{c}	19.18^{c}	27.72^{c}	51.42^{c}
DOGPO/Ox	13.77^{a}	21.22^{a}	34.86^{a}	42.85^{a}	86.00^{a}
DOGPO/Br	14.06^{a}	22.73^{a}	34.51^{a}	42.68^{a}	85.78^{a}
MOCAP	14.18^{a}	22.65^{a}	35.03^{a}	43.00^{a}	86.26^{a}
S.E.M	0.12	0.18	0.16	1.01	1.07
Treatment level (mg/ml)					
0	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}
15	1.22^{c}	4.24^{c}	7.03^{c}	12.48^{c}	23.37^{c}
25	4.03^{b}	9.14^{b}	14.29^{b}	18. 56^{b}	34.10^{b}
35	7.08^{a}	13.12^{a}	19.33^{a}	24.71^{a}	41.09^{a}
S.E.M	0.01	0.06	0.10	0.08	0.12

Means given column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 3. Effect of treatment and level of application of orange peel oils on egg hatching of *Pratylenchus penetrans*.

Treatments	Time of exposure (days)				
	1	2	3	4	5
FOGPO	0.00	0.00	0.00	0.00	0.00
FOGPO/Ox	0.00	0.00	0.00	0.00	0.00
FOGPO/Br	0.00	0.00	0.00	0.00	0.00
DOGPO	0.00	0.00	0.00	0.00	0.00
DOGPO/Ox	0.00	0.00	0.00	0.00	0.00
DOGPO/Br	0.00	0.00	0.00	0.00	0.00
MOCAP	0.00	0.00	0.00	0.00	0.00
S.E.M	0.00	0.00	0.00	0.00	0.00
	N.S	N.S	N.S	N.S	N.S
Treatment level mg/ml					
0	0.00^{a}	1.18^{b}	1.32^{b}	1.69^{b}	2.38^{b}
15	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
25	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
35	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
S.E.M	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}

Means in column followed by the same letter are not significantly different at $p \leq 0.05$.

There were significant differences in the activity of the orange oils; the decomposed brominated/oxidised oil significantly ($p \leq 0.05$) more effective than the fresh brominated/oxidised oils and compared well with the standard check mocap. The decomposed orange peel oil was effective than the fresh peel oil and showed 51.42% mortality as compared to 39.38% after 24 hrs. Plants received brominated/oxidised oils were taller with better yields than those in untreated and nematode infested plots in the field. Plants treated with the lowest concentration of oils 15 mg/ml were stunted and very low yields as compared to mocap. The highest population of nematode in the soil and root were observed in the inoculated untreated plots in the first and second trial (Table 4, 5, 6 and 7).

Table 4. Effect of treatment and level of application of orange peel oils and mocap on maize plant height (cm) under *Pratylenchus penetrans* infection in the field.

Treatments	5 wap*		7 wap		9 wap		13 wap	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
FOGPO	6.51[c]	8.12[c]	10.00[d]	12.55[c]	16.14[d]	20.13[d]	35.15[d]	39.27[d]
FOGPO/Ox	10.18[b]	11.88[b]	15.79[b]	18.68[b]	26.05[b]	29.11[b]	44.88[b]	49.59[b]
FOGPO/Br	10.07[b]	12.06[b]	16.22[b]	19.05[b]	25.83[b]	28.75[b]	45.20[b]	50.06[b]
DOGPO	7.26[c]	9.08[c]	12.18[c]	13.06[c]	20.41[c]	24.19[c]	39.56[c]	44.23[c]
DOGPO/Ox	12.94[a]	15.72[a]	20.59[a]	23.91[a]	30.81[a]	33.66[a]	50.06[a]	54.10[a]
DOGPO/Br	12.67[a]	15.86[a]	20.71[a]	24.00[a]	30.74[a]	34.07[a]	49.55[a]	53.61[a]
MOCAP	13.18[a]	16.03[a]	21.11[a]	23.87[a]	31.19[a]	33.71[a]	49.86[a]	54.18[a]
S.E.M	0.10	0.09	0.13	0.15	0.08	0.04	0.11	0.10
Treatment level mg/ml								
0	3.00[d]	4.28[d]	5.37[d]	7.22[d]	10.44[d]	12.06[d]	17.47[d]	19.21[d]
15	4.22[c]	6.42[c]	9.26[c]	11.06[c]	15.33[c]	18.49[c]	25.48[c]	29.74[c]
25	7.39[b]	9.28[b]	13.39[b]	15.27[b]	20.18[b]	23.45[b]	30.10[b]	33.00[b]
35	10.25[a]	11.31[a]	17.57[a]	19.65[a]	26.69[a]	29.51[a]	35.78[a]	38.22[a]
S.E.M	0.05	0.07	0.03	0.01	0.04	0.06	0.12	0.10

*Weeks after plantation

Means in column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 5. Effect of treatments and level of application of orange peel oils and mocap on number of days to 50% tasseling of maize plants under *Pratylenchus penetrans* infection on the field.

Treatments	Days to 50% tasselling	
	1st trial	2nd trial
FOGPO	67.37[d]	65.41[d]
FOGPO/Ox	56.55[b]	54.69[b]
FOGPO/Br	57.15[b]	55.03[b]
DOGPO	60.01[c]	60.19[c]
DOGPO/Ox	52.60[a]	50.84[a]
DOGPO/Br	53.00[a]	51.08[a]
MOCAP	52.76[a]	50.89[a]
S.E.M	0.22	0.18
Treatment level mg/ml		
0	58.15	61.22
15	47.29	51.14
25	41.08	44.21
35	35.00	38.09
S.E.M	0.15	0.19

Means in column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 6. Effect of treatments and level of application of orange peel oils and mocap on nematode populations of maize plants under *Pratylenchus penetrans* infection at harvest.

Treatments	Nematode population			
	250 g soil		15 g root sample	
	1st trial	2nd trial	1st trial	2nd trial
FOGPO	1.09[b]	2.04[b]	1.21[b]	1.17[b]
FOGPO/Ox	0.00[a]	0.00[a]	0.00[a]	0.00[a]
FOGPO/Br	0.00[a]	0.00[a]	0.00[a]	0.00[a]
DOGPO	0.15[a]	0.09[a]	0.02[a]	0.05[a]
DOGPO/Ox	0.00[a]	0.00[a]	0.00[a]	0.00[a]
DOGPO/Br	0.00[a]	0.00[a]	0.00[a]	0.00[a]
MOCAP	0.00[a]	0.00[a]	0.00[a]	0.00[a]
S.E.M	0.03[c]	0.01[c]	0.02[c]	0.01[c]
Treatment level mg/ml				
0	291.23[b]	254.56[b]	128.67[c]	102.78[c]
15	0.01[a]	0.03[a]	0.11[b]	0.13[b]
25	0.00[a]	0.00[a]	0.00[a]	0.00[a]
35	0.00[a]	0.00[a]	0.00[a]	0.00[a]
S.E.M	1.21	1.17	0.74	1.06

Means in a segment of a given column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 7. Effect of treatments and level of application of orange peel oils and mocap on yield of maize infected with *Pratylenchus penetrans* on the field.

Treatments	Yield (kg/ha^{-1}) 1st trial	Yield (kg/ha^{-1}) 2nd trial
FOGPO	33.63	36.16
FOGPO/Ox	44.57	48.76
FOGPO/Br	45.08	49.13
DOGPO	38.81	40.41
DOGPO/Ox	52.11	55.29
DOGPO/Br	52.00	55.07
MOCAP	51.75	54.81
S.E.M	1.15	1.11
Treatment level mg/ml		
0	10.36	12.58
15	18.43	22.47
25	25.38	28.54
35	31.46	34.29
S.E.M	0.14	0.17

Means in column followed by the same letter are not significantly different at $p \leq 0.05$.

Discussion

The major constituent of orange peel oil was limonene agreed with the findings of Karioti *et al*., (2007). Furthermore, Vekiari *et al*., (2004) reported limonene as the major compound found in citrus peel oils. Many of the essential oils (terpenes and terpenoids) have been reported to be nematicidal. The nematicidal activities of monoterpenoids on males, females and juveniles of the phytonematode *Bursaphelenchus xylophilus* was established by Choi *et al*., (2007). Larval development and egg hatching was inhibited at 0.33 mg/ml which was the lowest concentration of oil tested. Fabiyi *et al*., (2013) reported the toxicity of polar fractions from *Acanthus ilicifolius* on *Pratylenchus zea* juveniles. There was 73.67% mortality at 0.3 mg/ml concentration.

Conclusion

Orange peel oils (fresh and decomposed peels) have been found as effective as mocap in inhibiting reproduction of *P. penetrans* in the laboratory and field conditions. The products obtained through oxidation and bromination of these oils were significantly more effective than the ordinary fresh or decomposed orange peel oils alone, while the decomposed oil was found significantly effective than the fresh peel oil. Specific isolation and characterisation of the products of decomposition of the peel oil, oxidation and bromination of the orange peel oils and their utilization as bio-nematicides is intended as subject of future investigation.

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