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Nematicidal investigations of some essential oils from citrus plants

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ABSTRACT

Essential oils from citrus plants are getting increasing interest in the agricultural pest management. Numerous studies demonstrate their antibacterial, antifungal, insecticidal and nematicidal properties. This study was conducted to determine the activity of the essential oils from citrus plants (bergamot, bitter orange, grapefruit, mandarin, orange, and lemon) against the rhabditid nematode *Panagrolaimus* sp. In vitro nematicide screening was performed with aqueous solutions of essential oils in serial dilutions (1-0.0078%). The contact bioassay was done in small glass Petri dishes containing 3 ml of solution and 50 nematodes at 18°C. The experiment was carried out in five replicates. The lethal effect was monitored after 24h. Median lethal concentration (LC_{50}) was calculated using the Probit analysis. The best nematicidal activity performed the essential oil from lemon, followed by bergamot. The bitter orange had better nematicidal properties than mandarin and grapefruit essential oils. The least activity demonstrated orange oil.

Key words: essential oils, citrus, *Panagrolaimus*, nematodes

INTRODUCTION

Essential oils (EOs) of citrus plants are getting increasing interest due to their utility as alternatives to the hazardous chemical pesticides in the agricultural pest management. Some essential oils from citruses and their components are tested as anti-cancer drugs. Numerous studies demonstrate their antibacterial, antifungal, and insecticidal properties.

Sweet orange EO was reported to be efficient against several bacteria including *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* (Tao et al., 2009) and it was efficient against fungal species, such as *Alternaria alternata, Cladosporium herbarum, Curvularia lunata, Fusarium oxysporum, Helminthosporium oryzae, Penicillium chrysogenum,* and *Trichoderma viride* (Viuda-Martos et al., 2008; Singh et al., 2010).

Grapefruit EO showed antibacterial activity against *Paenibacillus larvae, Salmonella enteritidis, S. aureus, E. coli, Klebsiella pneumoniae, B. subtilis, Micrococcus luteus* (Gupta et al., 2011), and also showed a strong antifungal activity against *Aspergillus niger, Candida albicans, C. cucumerinum, Penicillium* spp. (Tirillini, 2000, Okunowo et al., 2013, Churata-Oroya et al, 2016). Grapefruit EO had insecticidal effect toward the mosquito *Aedes aegypti* (Ivoke et al., 2013). As anticancer drug was cytotoxic to human prostate and lung cancer cells (Zu et al., 2010).

Mandarin oil was efficient growth inhibitor of several bacteria including *E. coli, B. subtilis, Pseudomonas aeruginosa,* and *S. aureus* as well as phytopathogenic fungi including *A. alternata, Rhizoctonia solani, Curvularia lunata, F. oxysporum,* and *H. oryzae* (Chutia et al., 2009; Yi et al., 2018).

Bitter orange EO was acting as growth inhibitor of *Listeria innocua, S. enterica,* and *E. coli* (Friedman et al., 2004; Iturriaga et al., 2012) and performed the similar property against *Penicillium digitatum*, and *P. italicum* (Caccioni et al., 1998).

Bergamot EO exhibited antibacterial activity against *Campylobacter jejuni*, *E. coli* O157, *L. monocytogenes*, *B. cereus* and *S. aureus* (Fisher & Phillips, 2006), and antifungal activity against some dermatophytes including *Trichophyton* spp., *Microsporum* spp., and *Epidermophyton floccosum* (Sanguinetti et al., 2007). In addition, bergamot EO had antimycoplasmal activity against *Mycoplasma hominis, M. fermentans*, and *M. pneumoniae* (Furneri et al., 2012).

Lemon EO was inhibitory to *S. aureus, B. cereus, S. faecium, E. coli, S. typhi,* and *Shigella dysentery* (Hojjati & Barzegar, 2017). Lemon oil also had preservative effect against *L. monocytogenes* inoculated in minced beef meat (Hsouna et al., 2017). In experiments with *Erwinia amylovora* and *Pseudomonas syringae* pv. *syringae* both bacterial strains were sensitive, while *Xanthomonas campestris* pv. *campestris* was very sensitive to lemon EO (Popović et al., 2018). Lemon EO was effective as a repellent against *A. stephensi* (Oshagi et al., 2003), and as an acaricide against *Sarcoptes scabiei* (Aboelhadid et al., 2016). In addition, lemon EO showed cytotoxic effects on human prostate, lung, and breast cancer cells (Zu et al., 2010).

The nematicidal activity of EOs were determined against the plant parasitic nematodes: *Bursaphelenchus xylophilus*, *Meloidogyne* spp., *Ditylenchus dipsaci* (Park et al., 2005; Zouhar et al., 2009; Barbosa et al., 2010; Andres et al., 2012; Faria, 2015). The complex life cycle of parasitic nematodes, which rely on a host for propagation e.g. the potato cyst nematodes (Oro et al., 2014), make it challenging to examine a small molecule's impact on these animals. It was demonstrated that the free living rhabditid nematode *Caenorhabditis elegans* offered throughput that was not possible with parasitic species and represented a convenient alternative model system to search for new compounds that specifically kill nematodes (Burns et al., 2015).

The main aim of the study was to determine *in vitro* nematicidal activity of the essential oils from citrus plants (bergamot *Citrus bergamia* Risso, bitter orange *Citrus aurantium* L. ssp. amara, grapefruit *Citrus paradisi* Macf., mandarin *Citrus reticulata* Blanco, orange, *Citrus aurantium* L., and lemon *Citrus* × lemon (L.) Osbeck) against the free-living rhabditid nematode *Panagrolaimus* sp. Fuchs.

MATERIAL AND METHODS

Nematode culture

The panagrolaimid nematodes were grown as monoxenic culture on sterilized plant substrate and extracted with Baermann funnels (Hooper, 1986). The nematode culture old 24^h was used in all experiments.

Direct contact bioassay

The commercial essential oils from six citrus plants (bergamot, bitter orange, grapefruit, lemon, mandarin, and orange) were obtained to investigate their *in vitro* nematicidal activity against the rhabditid nematode *Panagrolaimus* sp. The experiment was performed with aqueous solutions of essential oils in serial dilutions (1%, 0.5%, 0.25%, 0.125%, 0.0625%, 0.0313%, 0.0156%, 0.0078% v/v) stabilized with 1% Break-Thru® 446 oil enhancer. The Direct contact bioessey was done in small glass Petri dishes containing 3 ml of solution and 50 nematodes incubated at 18°C. The experiment was carried out in five replicates. The lethal effect was monitored after 24h. Prior to the assessment of the EOs, the mortality of panagrolaimid nematodes in the control (the aqueous solution without EO) was compared with the mortality of nematodes in emulsifier and no significant differences between treatments were observed.

Data analysis

In order to evaluate nematicidal activity of citrus EOs the median lethal concentration (LC_{50}) was calculated using the Probit Analysis (Finney, 1952). The mortality of the exposed population of *Panagrolaimus* was corrected using the Abbott's formula (Abbott, 1985). Dose response curves were drawn using the $\log_{10}$ concentrations (x) and probits (y) and the regression equation (Y=intercept+slope×X) is derived with the help of MS Excel spreadsheets (McDonald, 2014).

RESULTS AND DISCUSSION

The median lethal concentration (LC_{50}) of the citrus EOs obtained from the Probit analyses varied from 0.224% (0.146-0.365) for orange EO to 0.049% (0.034-0.068) for lemon EO. Bergamot EO had the best nematicidal activity after the lemon EO with the LC_{50} of 0.057% (0.029-0.088). The bitter orange with LC_{50} of 0.121% (0.072-0.163) had better nematicidal properties than mandarin (LC_{50} of 0.147% (0.097-0.222)) and grapefruit (LC_{50} of 0.194% (0.083-0.342)) essential oils. The summary statistics followed by the dose (concentration) response relationships are presented in Tables1-6 and Figures 1-6, respectively.

Table 1. Probit analysis of orange EO on panagrolaimid nematode

Regression	*Statistics*	*Pred. Y*	*Residuals*
Multiple R	0.9910	6.1022	0.1127
R Square	0.9820	5.5940	−0.0470
Adjusted R Square	0.9775	5.0858	−0.1844
Standard Error	0.1438	4.5775	0.0018
Observations	6	4.0693	0.1746
		3.5622	−0.0576
ANOVA	*df*	*SS*	*F*
Regression	1	4.5174	218.3755
Residual	4	0.0827	*Significance F*
Total	5	4.6001	0.0001
	Coefficients	*Stand. Error*	*P-value*
Intercept	−0.6511	0.3756	0.1581
X Variable 1	1.6883	0.1142	0.0001

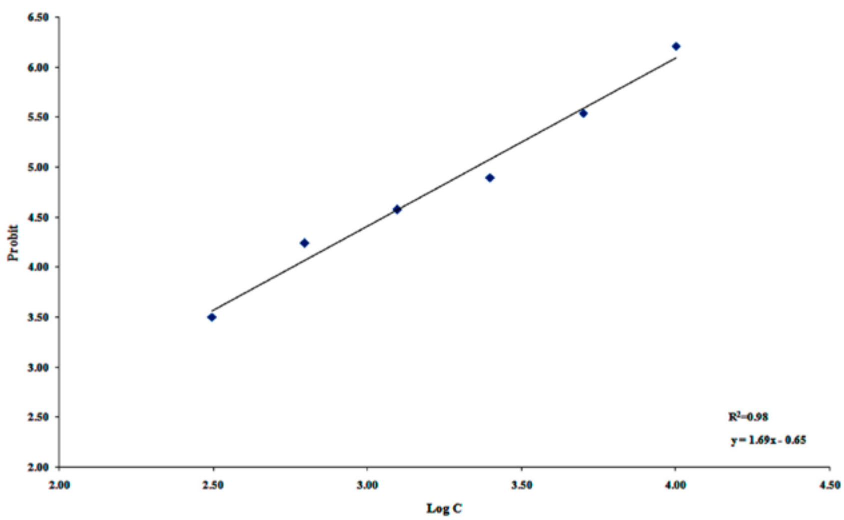

Figure 1. Dose response relationship of orange EO on panagrolaimid nematode

Table 2. Probit analysis of grapefruit EO on panagrolaimid nematode

Regression	*Statistics*	*Pred. Y*	*Residuals*
Multiple R	0.9814	6.1455	−0.0706
R Square	0.9631	5.6558	−0.0801
Adjusted R Square	0.9508	5.1661	0.2112
Standard Error	0.1750	4.6763	0.1002
Observations	5	4.1866	−0.1607
ANOVA	*df*	*SS*	*F*
Regression	1	2.3983	78.3236
Residual	3	0.0918	*Significance F*
Total	4	2.4901	0.0030
	Coefficients	*Stand. Error*	*P-value*
Intercept	−0.3618	0.6295	0.6057
X Variable 1	1.6268	0.1838	0.0030

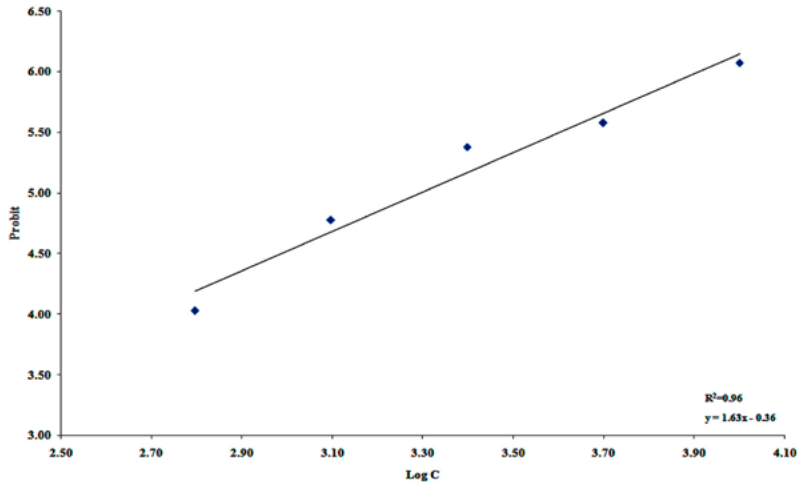

Figure 2. Dose response relationship of grapefruit EO on panagrolaimid nematode

Table 3. Probit analysis of mandarin EO on panagrolaimid nematode

Regression	*Statistics*	*Pred. Y*	*Residuals*
Multiple R	0.9920	6.3833	0.1060
R Square	0.9840	5.8805	−0.1700
Adjusted R Square	0.9808	5.3777	−0.0591
Standard Error	0.1516	4.8749	0.0108
Observations	7	4.3721	0.2485
		3.8704	−0.0786
		3.3653	−0.0575
ANOVA	*df*	*SS*	*F*
Regression	1	7.0801	307.83
Residual	5	0.1150	*Significance F*
Total	6	7.1951	1.1028E-05
	Coefficients	*Stand. Error*	*P-value*
Intercept	−0.2979	0.3003	0.3668
X Variable 1	1.6703	0.0952	1.1028E-05

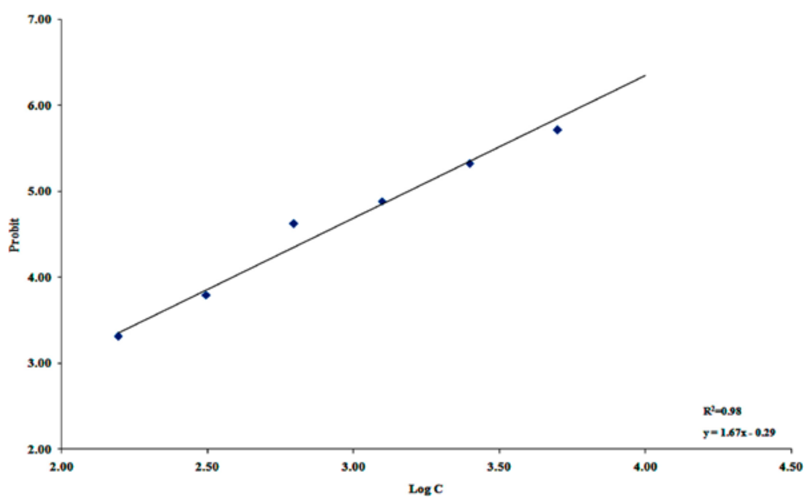

Figure 3. Dose response relationship of mandarin EO on panagrolaimid nematode

Table 4. Probit analysis of bitter orange EO on panagrolaimid nematode

Regression	*Statistics*	*Pred. Y*	*Residuals*
Multiple R	0.9704	6.2728	−0.2445
R Square	0.9416	5.6548	0.1499
Adjusted R Square	0.9222	5.0368	0.3461
Standard Error	0.2808	4.4187	−0.1639
Observations	5	3.8021	−0.0877
ANOVA	*df*	*SS*	*F*
Regression	1	3.8160	48.3868
Residual	3	0.2366	*Significance F*
Total	4	4.0526	0.0061
	Coefficients	Stand. Error	P-value
Intercept	−1.3212	0.9226	0.2475
X Variable 1	2.0530	0.2951	0.0061

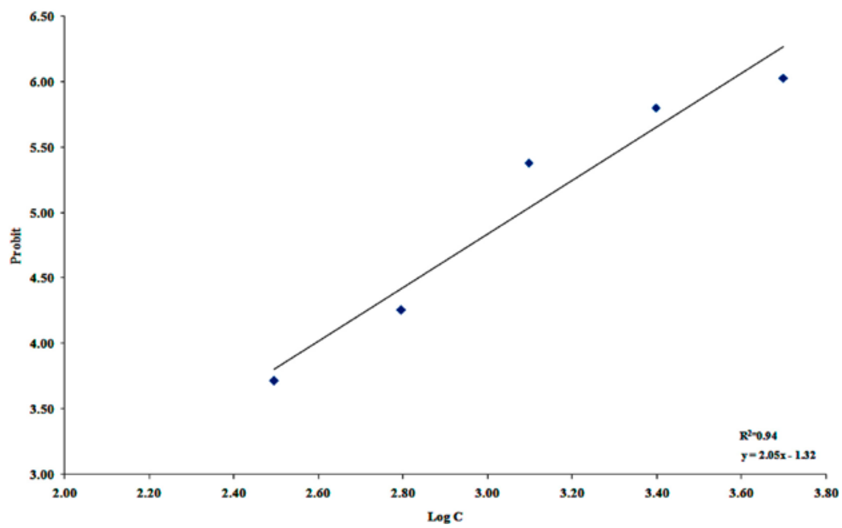

Figure 4. Dose response relationship of bitter orange EO on panagrolaimid nematode

Table 5. Probit analysis of bergamot EO on panagrolaimid nematode

Regression	*Statistics*	*Pred. Y*	*Residuals*
Multiple R	0.9817	6.4425	0.1388
R Square	0.9637	5.7626	−0.1239
Adjusted R Square	0.9456	5.0828	−0.1688
Standard Error	0.2084	4.4044	0.1540
Observations	4		
ANOVA	*df*	*SS*	*F*
Regression	1	2.3081	53.1550
Residual	2	0.0868	*Significance F*
Total	3	2.3949	0.0183
	Coefficients	*Stand. Error*	*P-value*
Intercept	−1.2319	0.9187	0.3119
X Variable 1	2.2586	0.3098	0.0183

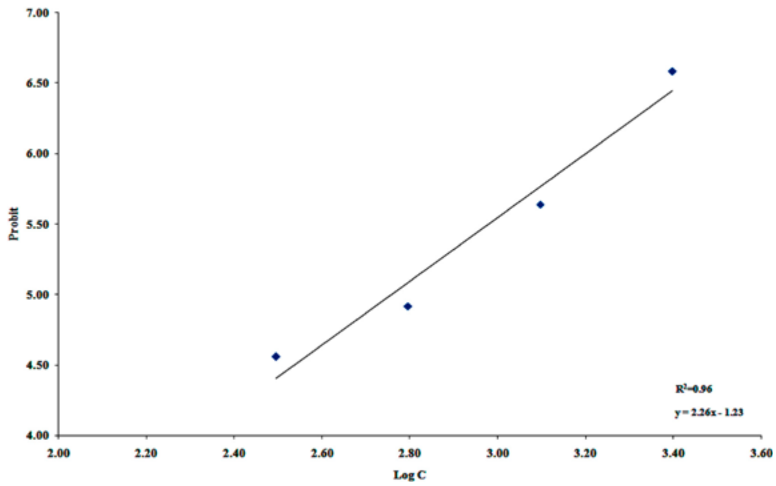

Figure 5. Dose response relationship of bergamot EO on panagrolaimid nematode

Table 6. Probit analysis of lemon EO on panagrolaimid nematode

Regression	*Statistics*	*Pred. Y*	*Residuals*
Multiple R	0.9983	7.0645	–0.9949
R Square	0.9965	6.4498	0.1168
Adjusted R Square	0.9958	5.8352	0.0538
Standard Error	0.0861	5.2205	–0.0812
Observations	7	4.6073	–0.0699
		3.9898	0.0541
		3.3751	0.0047
ANOVA	*df*	*SS*	*F*
Regression	1	10.5857	1427.1470
Residual	5	0.0371	*Significance F*
Total	6	10.6228	2.4484E-07
	Coefficients	*Stand. Error*	*P-value*
Intercept	–0.4883	0.1546	0.0251
X Variable 1	2.0419	0.0540	2.4484E-07

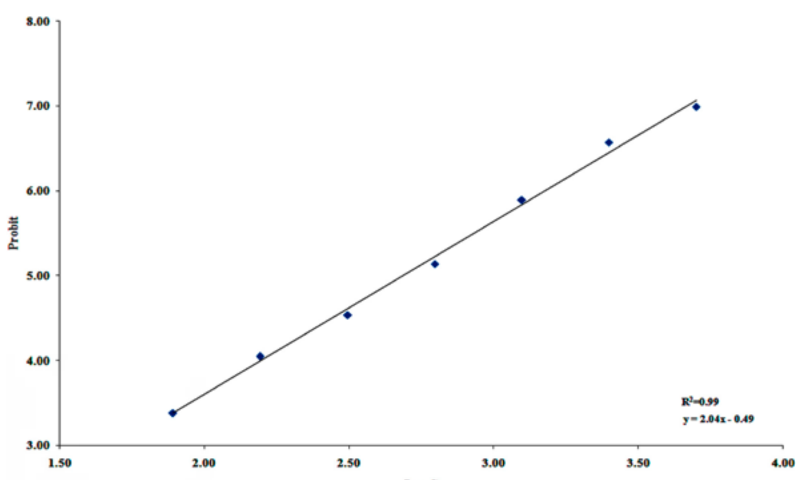

Figure 6. Dose response relationship of lemon EO on panagrolaimid nematode

The dose (concentration) response relationships show a strong relation between the nematode mortality and the concentration of the examined oils. All regression coefficients are close to 1 indicating a homogenous population of nematodes. Beside lemon and bergamot EOs which performed moderate efficiency, all other EOs were slightly toxic to *Panagrolaimus* sp. The results of this study are congruent with the similar studies performed with plant parasitic nematodes (Park et al., 2005; Kong et al., 2006). In the study of Park et al. (2005) the nematicidal activity of *Citrus limonia* EO in the concentration of 2000 µl/l caused mortality of 69.7% of juveniles of *B. xylophilus*. The concentration of 10 mg/ml of the lemon EO was lethal to 86% of the population of *B. xylophilus* (Kong et al., 2006). In our study, the concentration of 0.5% of lemon and bergamot EOs caused mortality of 97.65% and 100% of the panagrolaimid nematodes with the strongest nematicidal effect. In addition, we also demonstrated that the results for the acute toxicity of the examined EOs were comparable with the similar studies based on plant parasitic nematodes suggesting that the panagrolaimid nematode can be used as a model organism for toxicity testing.

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