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Nematicidal and larvicidal activities of the essential oils from aerial parts of *Pectis oligocephala* and *Pectis apodocephala* Baker

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ABSTRACT

The chemical composition of the essential oils from aerial parts of Pectis apodocephala and *Pectis oligocephala* were analyzed by GC-MS. The essential oils of these species were predominantly constituted by monoterpenes. Geranial (42.9–44.5%), neral (32.2–34.2%) and α-pinene (10.7–11.4%) were the main constituents in the oil of *P. apodocephala*, while *p*-cymene (50.3–70.9%) and thymol (24.4–44.7%), were the prevalent compounds in the oil of *P. oligocephala*. The essential oils were tested against the root knot nematode *Meloidogyne incognita* and *Aedes aegypti* larvae survival. The results obtained show that both essential oils exhibited significant activity and could be considered as potent natural namaticidal and larvicidal agents.

Key words: *Pectis apodocephala*, *Pectis oligocephala*, essential oil, nematicidal activity, larvicidal activity.

INTRODUCTION

Essential oils from different plant sources have demonstrated several biological activities, including antibacterial (Iacobellis et al. 2005), insecticidal (Araújo et al. 2003), larvicidal (Albuquerque et al. 2004a), acaricidal (Kim et al. 2004), nematicidal (Duschatzky et al. 2004) and antifungal (Wang et al. 2005). As a consequence, this vast arsenal of bioactive compounds has attracted significant and crescent attention of researchers in recent years. Nematodes are tiny worms, some of them are parasites to plants, and can play an important role in the predisposition of the host plant to the invasion by secondary pathogens (Jayasinghe et al. 2003). Plants attacked by nematodes show retarded growth and development, as well as loss in the quality and quantity of the harvest product. The nematode *Meloidogyne incognita* is a widespread nematode found in several important agronomical cultures of Brazil.

Innumerous human diseases such as malaria, filariasis, dengue fever, yellow fever and several viral diseases are transmitted by mosquitoes (Venkatachalam and Jebanesan 2001), being the *Aedes aegypti* one of the most hazardous because it is the vector for yellow fever and dengue hemorrhagic fever, besides to be responsible for some allergic response such as urticaria and angioedema (Cheng et al. 2003). The synthetic pesticides have been generally used with success for the eradication of nematode *M. incognita* and *A. aegypti* larvae. Nevertheless, the continuous and indiscriminate use of synthetic in-

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secticides has also induced undesirable effects including toxicity to non-target organisms and environmental pollution problems. Since essential oils constitute a rich source of bioactive compounds, they may be considered as an useful alternative strategy to the currently available insecticidal agents in the use for controlling both *M. incognita* and *A. aegypti*.

As part of a continuous research program on aromatic plants from Northeast Brazil, we have investigated the volatile composition of the essential oils of several plants from the Asteraceae family (Albuquerque et al. 2001, 2003, 2004a, b). The genus Pectis is constituted by approximately 76 neotropical species. Despite the etnobotanical uses of some of them, this genus has been the subject of scant phytochemical and biological studies. In the flora of Northeast Brazil, the genus is represented by five species, including *Pectis apodocephala* and *Pectis oligocephala*, whose essential oils we have previously investigated (Craveiro et al. 1986, Albuquerque et al. 2003). The present work reports the reinvestigation of the essential oils from aerial parts of *P. apodocephala* and *P. oligocephala*, as well as their nematicidal and larvicidal effects against the nematode *M. incognita* and *A. aegypti* larvae.

MATERIALS AND METHODS

Plant Material

Both *P. apodocephala* and *P. oligocephala*, in the flowering stage were collected in May 2000 and June 2003, in Sobral County, State of Ceará, Northeast Brazil. Voucher specimens (#29441 and #29453) were deposited at the Herbário Prisco Bezerra (EAC) of the Departamento de Biologia, Universidade Federal do Ceará, Brazil.

Extraction of the Essential Oils

Samples of fresh aerial parts of both species were subjected to hydrodistillation for 2 hours in a Clevenger-type apparatus. The isolated oils were subsequently dried over anhydrous sodium sulfate and stored under refrigeration until analyzed and tested. The oil yields (w/w on fresh weight basis) for *P. apodocephala* were 0.20 and 0.12%, while the oil yields for *P. oligocephala* were 0.40 and 0.18%, respectively.

Analysis of the Essential Oils

The volatile components from aerial parts of *P. apodocephala* and *P. oligocephala* (Table I), were analyzed by GC-MS on a Hewlett-Packard Model 5971 GC/MS using a (5%-phenyl)-methylpolysiloxane DB-5 capillary column (30 m $\times$ 0.25 mm) with film thickness 0.1μm; carrier gas helium, flow rate 1 mL/min with split mode. The injector temperature and detector temperature were 250°C and 280°C, respectively. The column temperature was programmed at 4°C/min from 35°C to 180°C and then at 10°C/min from 180°C to 250°C. Mass spectra were recorded from 30–450 m/z. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer data base using the Wiley L-Built library and two other computer library MS searches using retention indices as a preselection routine (Alencar et al. 1984, 1990) as well as by visual comparison of the fragmentation pattern with those reported in the literature (Stenhagen et al. 1974, Adams 2001).

Used Organisms

Second-stage juvenile (J2) of the plant parasite root-knot nematode *Meloidogyne incognita* and instar III larvae of *Aedes aegypti*.

Nematicidal Bioassay

Egg masses of *M. incognita* obtained from okra roots with aid of a stereomicroscope were maintained in Petri dishes during 24 h in distilled water for the juveniles eclosion. 50μL portions of water containing approximately 100 juveniles (J2) were transferred to nematological vials to which 1 mg of the oils to be tested was added and a H_2O:DMSO 2% solution was poured to complete 1 mL. The nematological vials were kept on a hood at 28°C. The inactive nematodes counting were performed at every 24 h for 72 h. After the last counting the inactive juveniles were maintained in distilled water for 24 h to observe their revival. Four repetitions for each treatment were performed using water and H_2O:DMSO 2% solution as control. Table II shows the nematicidal effect for the essential oils from aerial parts of *P. apodocephala* and *P. oligocephala*. In Table II the survival figures mean the number of juveniles showing motionless that recovered motility after maintained 24 h in fresh water.

TABLE I
Volatile components identified in the essential oils from aerial parts of *P. apodocephala* (oils I and I') and *P. oligocephala* (oils II and II').

Compounds[a]	Relative contents (%)				
	IK[b]	oil I[c]	oil I'[d]	oil II[c]	oil II'[d]
α-pinene	940	11.4	10.7	–	0.2
6-methyl-5-hepten-2-one	991	1.0	–	–	–
myrcene	993	–	–	1.7	0.7
α-phellandrene	1007	–	–	0.9	0.5
limonene	1034	6.7	6.9	–	1.2
p-cymene	1026	–	–	70.9	50.3
β-phellandrene	1046	–	–	2.1	–
nerol	1221	–	0.8	–	–
neral	1235	34.2	32.2	–	–
geraniol	1252	1.3	3.4	–	–
geranial	1270	44.5	42.9	–	–
thymol	1289	–		24.4	44.7
β-caryophyllene	1427	–	0.6	–	0.4
α-humulene	1456	–	0.6	–	–
Total identified		99.1	98.1	100.0	98.0

[a]Order of elution on DB-5 capillary column. [b]IK = Kovats retention index in reference to C_8–C_{26} n-alkanes on DB-5 column. [c]Samples of *P. apodocephala* and *P. oligocephala* collected in May 2000. [d]Samples of *P. apodocephala* and *P. oligocephala* collected in June 2003.

TABLE II
Nematicidal activity (%) of the essential oils from aerial parts of *P. apodocephala* (oil I and I') and *P. oligocephala* (oil II and II') expressed as the average of four repetitions for each treatment.

Hours	Larval mortality (%)					
	oil I	oil I'	oil II	oil II'	Control	H_2O
24	90	81	96	92	0	0
48	92	90	100	97	2	4
72	94	92	100	98	6	9
Survival*	0	0	0	0	6	8

*Number of motionless juveniles that recovered motility.

Larvicidal Bioassay

Portions of essential oils (5 to 500μg/mL) were placed in a beaker (50 mL) and dissolved in H_2O:DMSO (98.5:1.5). 50 instar III larvae of *A. aegypti* were delivered to each beaker. After 24 hours, at room temperature, the number of dead larvae was counted and the lethal percentage calculated. A control using DMSO and water was carried out in parallel. For each sample, three independent experiments were run. The bioassays were performed at Laboratório de Entomologia, Núcleo de Endemias da Secretaria de Saúde do Estado do Ceará, Brazil.

RESULTS AND DISCUSSION

Table I shows the identified constituents and their percentage composition, as well as their Kovats Indices (KI) values listed in order of elution from a DB-5 capillary column. The chemical composition of the essential oils from both species, *P. apodocephala* and *P. oligocephala*, were essentially constituted by monoterpenes, but as can be seen from Table I, the essential oil of each species has a characteristic chemical composition, either qualitative or quantitative, that has been maintained steady independent of the time of extraction. The main compounds of the oil of *P. apodocephala*, in order of their abundance, were: geranial (42.9–44.5%) and neral (32.2–34.2%) followed by α-pinene (10.7–11.4%) and limonene (6.7–6.9%). On the other hand, the most prevalent compounds detected in the oils of *P. oligocephala* were *p*-cymene (50.3–70.9%) and thymol (24.4–44.7%).

The nematicidal and larvicidal potential of the oils from both species were evaluated against the nematode *M. incognita* and the *A. aegypti* larvae. The results of the bioassays are summarized in Tables II and III. Since all oils from both plants exhibited similar and significant activities in the bioassays and the oils have very different chemical composition this make evident the unspecific susceptibility of the tested organisms. Several papers recently published have dealed with biological activities of essential oils. Citral (geranial + neral) rich oils presented antifungal activity (Wang et al. 2005, Pattnaik et al. 1997, Belletti et al. 2004) while thymol rich oils presented, antibacterial, antifungal and larvicidal activities (Singh et al. 2004, Shin and Kim 2004, Tepe et al. 2004, Goren et al. 2003, Carvalho et al. 2003). Interestingly, *p*-cymene in particular, and other monoterpenoids such as α- and β-pinene are also present as the major components in all those biologically active oils. Despite the two-fold higher concentration of thymol in oil II' the activity of this oil is still similar to the one of oil II. On the other hand, the figures are inverted in the case of *p*-cymene concentrations. If at a lower concentration of *p*-cymene oil II' still showed a slightly improved activity this maybe means that *p*-cymene contributes more for the activity than thymol, once the total concentration of both components (95.3% and 95.0%, for oil II and II', respectively) is maintained steady. It is also worthy of notice the higher toxicity of oils II and II' relative to oils I and I' in both assays.

The steady chemical constitution pattern of the oils, associated to their nematicidal and larvicidal properties, could be relevant points to be considered to the possible economical potential of the oils from both species.

TABLE III
Larvicidal activity of the essential oils from leaves of *P. apodocephala* (oil I') and *P. oligocephala* (oil II').

Conc. (μg/mL)	Larval mortality (%)	
	oil I´	oil II´
500	96	100
250	62	100
100	22	100
50	3	60
25	Nt	17

nt = not tested.

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RESUMO

A composição química dos óleos essenciais das partes aéreas de *Pectis apodocephala* e *Pectis oligocephala* foi analisada por CG-EM. Os óleos essenciais destas espécies foram predominantemente constituídos por monoterpenos. Geranial (42,9–44,5%), neral (32,2–34,2%) e α-pineno (10,7–11,4%) foram os constituintes majoritários no óleo de *P. apodocephala*, enquanto *p*-cimeno (50,3–70,9%) e timol (24,4–44,7%), foram os compostos prevalentes no óleo de *P. oligocephala*. Ambos os óleos foram testados contra o nematóide *Meloidogyne incognita* e larvas do mosquito *Aedes aegypti* no terceiro estágio. Os resultados obtidos mostraram que os óleos exibem significante atividade e podem, portanto, ser considerados como potenciais agentes nematicida e larvicida naturais.

Palavras-chave: *Pectis apodocephala*, *Pectis oligocephala*, óleo essencial, atividade nematicida, atividade larvicida.

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