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Short communication

Chemical composition and larvicidal activity of essential oils from *Peganum harmala*, *Nepeta cataria* and *Phellodendron amurense* against *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT

Essential oils from aerial parts of the herbs *Peganum harmala* and *Nepeta cataria*, and leaves of the tree *Phellodendron amurense* were analyzed by GC-FID and GC-MS, and their larvicidal activities were assayed on the early fourth instar larvae of *Aedes aegypti*. The major constituents of the oils were limonene (14.5%) and thymol (11.5%) in *P. harmala*, thymol (46.5%), 4aα,7α,7aβ-nepetalactone (18.3%) and 4aα,7β,7aα-nep talactone (19.7%) in *N. cataria*, eugenol (14.5%) andγ-eudesmol (9.5%) in *P. amurense*.The oil of *N. cataria* had a strong larvicidal activity (LC_{50} < 50 μg/mL; LC_{90} < 86.8 μg/mL) on *A. aegypti* while the remaining oils showed a moderated killing effect. The larvicidal activity of *N. cataria* oil was associated to the contents of 1,8-cineol, camphor, 4aα,7α,7aβ-Nepetalactone, 4aα,7β,7aα-Nepetalactone and thymol. Our results indicate that the oil of *N. catariadeserves* to be used as a source of larvicidal agents against *A. aegypti*.

1. Introduction

The yellow fever mosquito (*Aedes aegypti* L., Culicidae) is the vector of many diseases such as the dengue fever, Zika fever, chikungunya, and malaria (ECDC, 2017). The eradication of the mosquito is the only way to fight against these diseases and is currently based on the continued application of organophosphates such as temephos and fenthion or pyrethroids like permethrin and resmethrin, insect growth regulators such as diflubenzuron and methoprene, and bacterial larvicides such as *Bacillus thuringiensis* and *B. sphaericus* (Isman, 2006). However, these control strategies led to the appearance of resistance in the larvae of the yellow fever mosquito and have a negative impact on non-target organisms disrupting natural biological control systems (Perry et al., 1998). The low biodegradability of larvicidal insecticides also showed an adverse long-term impact on human health. The use of essential oils or their constituents might aid to overcome these problems. Several of them showed a low mammalian toxicity, a selective activity against insect pests, and a short persistence in the environment (Dias and Moraes, 2014). The essential oils and their constituents usually exert their action at multiple levels in the target organisms, so there is a low likelihood of insect resistance (Sampietro et al., 2017).

There are 10,608 medicinal higher plants growing in China (Asgarpanah et al., 2013). This number is about 30% of all medicinal plant known in the world and constitute 83.4% of all the medicinal biological resources of the country. Some of them are readily available including esfand (*Peganum harmala* L., Nitrariaceae), catnip (*Nepeta cataria* L., Lamiaceae) and amur cork (*Phellodendron amurense* Rupr., Rutaceae). These plants are widely used to treat a variety of disorders, already indicating the presence of bioactive compounds. The aerial parts of *P. harmala* are used for the treatment of pain, skin inflammations and skin cancers as well as an emmenagogue and abortifacient agent (Dastagir et al., 2014).

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The dried leaves and flowering tops of *N. cataria* are administered as a tonic, carminative, and diaphoretic medicine and for infantile colic (Grognet, 1990). *Phellodendron amurense* is applied against meningitis, bacillary dysentery, pneumonia, tuberculosis, and liver cirrhosis (Lis et al., 2004). Essential oils from aerial parts of these plants were reported with insecticidal activity, although they were not assayed for their larvicidal activity on *A. aegypti*. For example, the oil from aerial parts of *P. harmala* showed contact toxicity for the third instar larvae of *trogoderma granarium* (Eltahir and Dahab, 2019). The oil from flower tops of *N. cataria* was insecticidal to the third instar of *S. littoralis* larvae after topical application, and had a repellent activity on a wide number of insects (Asgarpanah et al., 2013). The fruit oil of *P. amurense* had contact toxicity against *Musca domestica*, L. (Read and Zasada, 2008).

In light of the above knowledge, the aim of this work was to investigate the composition and the associated larvicidal activity of the essential oils from aerial parts of *P. harmala*, *N. cataria* and *P. amurense* against *A. aegypti*.

2. Materials and methods

2.1. Plant material

The aerial parts (leaves and stems) of *P. harmala* and *N. cataria* were collected in July 2014, from Alxa League of Inner Mongolia Autonomous Region, China (43.24°N latitude; 118.33° E longitude). Leaves of *P. amurense* were collected during July 2014, from the suburbs of Changchun city, Jilin Province, China (44.06°N latitude; 125.29° E longitude). The plants were identified by Prof. Ying Wu, College of Plant Science, Jilin University. Voucher specimens (2014022, *P. harmala*; 2014203, *N. cataria*; and 2014063, *P. amurense*) were deposited.

2.2. Extraction of the essential oils

The fresh plant materials (400 g) of *P. harmala*, *N. cataria* and *P. amurense* were subjected to hydrodistillation for 6 h using a Clevenger-type apparatus. The obtained essential oils were dried over sodium sulfate anhydrous and stored at 0 °C after filtration. Total oil yields were expressed based on dry weight of the plant material.

2.3. Analysis of the chemical composition of the essential oils

Essential oil samples were injected into a TRACE GC-FID for profiling analysis as well as a Thermo TRACE GC ULTRA-ITQI 100 GC-MS (70 eV) for identification of distinct compounds. Analyses were carried out on a PE-5 MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness). The GC-MS (70 eV) analysis was carried out on a Thermo TRACE GC ULTRA-ITQI 100, equipped with PE-5 MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness). The temperature was programmed 60 °C (3 min), then 60–220 °C at 8 °C min^{-1}, and finally 280 °C (20 min). The temperature of the injector was kept at 280 °C; the injection volume of each sample was 1 μL (diluted to 1:100 in acetone); the split ratio was 30:1. The carrier gas was helium at a flow rate of 1 ml min^{-1}. The MS source temperature was kept at 230 °C. The interface temperature was held at 280 °C. The spectra scan range was of 35–450 amu. The compounds were identified by comparison of their retention indices (RI) and mass spectra with those stored at the National Institute of Standards and Technology (Stein, 1990) and published data (Adams, 2007). Retention indices were calculated in relation to a homologous series of *n*-alkanes (C8-C26) injected under the same operating conditions. The percentage of participation of each compound was calculated based on peak-areas from the GC-FID profiling.

2.4. Bioassays

Aedes aegypti was originally obtained from the Chinese Center for Disease Control and Prevention. Anhydrous eggs of *A. aegypti* were hatched in glass trays filled with tap water. Larvae of *A. aegypti* were further cultivated in tubes on larval food (ground dog biscuits and yeast tablets in a relationship 1:1, w/w) until the fourth instar stage. The larvicidal activity was evaluated according to the larval susceptibility assay suggested by the World Health Organization (World Health Organization, 1981). Aqueous suspensions of the essential oils were prepared at concentrations comprised between 150.0 and 9.0 μg/mL. They were supplied with 0.05% DMSO as emulsifier. Suspensions were poured in 400 ml beakers by adding 250 ml per beaker. Each aqueous suspension was poured in five beakers and 20 larvae were placed in each beaker. Clorpyrifos obtained from the National Center of Pesticide Standards (Shenyang, China), and eugenol and thymol purchased in Sigma-Aldrich (China) were included as positive standards of larvicidal activity. Clorpyrifos was assayed in the range 0.3–5 μg/mL while eugenol and thymol were tested in the range 9–150 μg/mL. DMSO at 0.05% in tap water was assayed as negative control. The assays were incubated in glass growth jars (L26: D17) at 27–29 °C, with 78–80% relative humidity, and the number of dead larvae was recorded after 24 h. Mortality was absent in the controls. Hence, corrections of treatment mortalities based on the formula of Abbot were not needed (Abbott, 1925). Then, mortality values were used to calculate the concentration required to kill 50% (LC_{50}) and 95% (LC_{95}) of the larvae with the corresponding 95% confidence intervals. These calculations were done with Probit analysis provided by SPSS 13.0 for Windows (SPSS Inc., 2004). The LC_{50} values and the relative contents of the main constituents of the essential oils were also subjected to principal component analysis performed by XLSTAT v. 2009.3.02.

3. Results and discussion

3.1. Analysis of the essential oils

The aerial parts yielded oils (w/w) in 0.75% (*P. harmala*), 1.85% (*N. cataria*) and 1.25% (*P. amurense*). Oil yields were not previously reported for aerial parts of *P. harmala*. The oil yield obtained for *N. cataria* was higher than those previously reported which were in the range 0.11–1.50% (Reichert et al., 2016; Said-Al Ahl et al., 2018). The same is observed for the *P. amurense* leaves which previously showed a yield of 0.03% (Lis et al., 2004). The GC-MS analysis identified 21, 15 and 23 compounds of *P. harmala*, *N. cataria* and *P. amurense*, respectively, accounting for more than 76% of the chemical composition of the oils (Table 1). The oil of *P. harmala* contained mainly limonene (14.5%)and thymol (11.5%) while the oil of *N. cataria* had high contents of thymol (46.5%), 4aα,7α,7aβ-nepetalactone (18.3%) and 4aα,7β,7aα-nepetalactone (19.7%). Eugenol (14.5%) andγ-eudesmol (9.5%) were the most abundant constituents in the leaf oil of *P. amurense*. The remaining compounds were in contents below of 8%. Compositional variations in the oil from leaves of *N. cataria* have been mainly associated to changes in the genetic background and in a lesser extent to collecting season and location (Reichert et al., 2016; Asgarpanah et al., 2013). Three patterns of major constituents have been reported for oils of *N. cataria*. The first one shows only nepetalactones stereoisomers, the second contains these compounds together with high levels of monoterpenes such as 1,8-cineole, β-caryophyllene, α-pinene, geranyl acetate and/or α-humulene, and the third is free

Table 1
Compounds and their relative abundance from *Peganum harmala*, *Nepeta cataria* and *Phellodendron amurense*.

Compound[a]	RIcalc[b]	RI[c]	Relative area (%)[d]		
			P. harmala	*N. cataria*	*P.amurense*
Pyridine	751	757	2.8	–	–
trans-2-Hexenal	849	846	2.2	–	–
Hexanoic acid	971	967	2.0	–	–
Phenol	986	992	–	–	5.2
Limonene	1024	1024	14.5	0.6	1.1
1,8-cineol	1027	1026	–	1.0	–
Benzyl alcohol	1031	1026	–	–	7.9
α-Phenethyl alcohol	1060	1057	2.4	–	5.7
4-Methyl-phenol	1077	1071	–	–	4.6
Linalool	1096	1095	7.5	0.9	–
Camphor	1139	1141	–	0.6	–
4-Ethyl-phenol	1165	1168	–	0.4	1.8
trans-Linalool oxide	1174	1173	–	–	1.8
Octanoic acid	1270	1278	2.6	–	–
Thymol	1281	1289	11.5	46.5	1.7
Indole	1288	1290	3.4	–	–
4-vinylguaiacol	1312	1309	–	–	3.1
4′-Methoxyacetophenone	1340	1347	3.3	0.5	–
Eugenol	1356	1356	–	–	14.5
Decanoic acid	1366	1364	3.8	–	–
4aα,7α,7aβ-Nepetalactone	1385	1389	–	18.3	–
4aα,7β,7aα-Nepetalactone	1392	1392	–	19.7	–
Methyl eugenol	1406	1403	–	–	1.7
α-Bulnesene	1504	1509	–	–	3.0
trans-Nerolidol	1568	1564	–	–	2.6
Dodecanoic acid	1571	1570	5.8	–	–
Caryophyllene oxide	1583	1580	–	–	1.5
γ-Eudesmol	1633	1630	–	–	9.5
β-Eudesmol	1657	1650	–	–	3.2
α-Eudesmol	1660	1652	–	–	1.3
Myristic acid	1775	1780	2.8	0.3	–
Phytol	1940	1942	2.7	0.2	7.8
Palmitic acid	1973	1976	3.1	1.7	–
Eicosane	1997	2000	–	–	1.5
Linoleic acid	2134	2132	5.0	–	2.1
Monoterpene hydrocarbons			14.5	0.6	1.1
Oxygenated monoterpenes			7.5	40.5	1.8
Sesquiterpene hydrocarbons			–	–	3.0
Oxygenated sesquiterpenes			–	–	21.1
Fatty acid derivatives			28.3	2.0	3.6
Phenolic compounds			11.5	46.9	32.6
Miscellaneous			14.6	0.7	21.4
Total			76.4	90.7	84.6

[a] Compounds listed based on elution from a non-polar PE-5 column.
[b] Retention index calculated from retention times in relation to those of a series of *n*-alkanes (C8-C26) on a 30 m PE-5 capillary column.
[c] Retention Index taken from Stein (1990) and Adams (2007).
[d] Relative area (%): percentage of the area occupied by the compound within the chromatogram.; (–) without compound.

of nepetalactones (Asgarpanaha et al., 2013; Reichert et al., 2019; Said-Al Ahl et al., 2018). Our leaf oil belonged to the second one and is the first *N. cataria* oil informed with a high richness of both thymol and the nepetalactone stereoisomers. In the case of *P. harmala* and *P. amurense*, the composition of their oils have been scarcely explored and was completely different from those informed here. There is only one report on an oil obtained from aerial parts of *P. harmala* collected in Pakistan which showed high contents of camphor (28.2%) and capillin (13.2%) (Dastagir et al., 2014). Regarding the leaf oil of *P. amurense*, a sample from Poland was reported with β-elemol (18.5%), (Z)-β-ocimene (12.6%) and limonene (12.0%) as the main constituents (Lis et al., 2004).

3.2. Larvicidal activity and its relationship with the composition of the essential oils

Table 2 shows the larvicidal activity of the essential oils, the oxygenated monoterpene thymol, the phenylpropanoid eugenol and the organophosphate insecticide chlorpyrifos on the early fourth instar larvae of *A. aegypti*. The LC_{50} values of thymol (37.1 μg/ml) and eugenol (19.8 μg/ml) were below those available in the literature which were informed in the range 46.0–59.8 μg/ml and 60.9–93.3 μg/ml, respectively. This fact may be due to methodological differences in the larvicidal assay and/or a lower susceptibility to thymol and eugenol of the *A. aegypti* strains assayed (Barbosa et al., 2012; Barros Silva et al., 2017; Pandiyan et al., 2019). The larvicidal activity of both compounds strongly remains in their passage through the larvae cuticle which is due to the hydrophobic groups of their molecules (Santos et al., 2011). Based on Dias and Moreaes (2014), an essential oil or an oil constituent can be a strong (LC_{50} < 50 μg/ml), a moderate (50 μg/ml < LC_{50} < 100 μg/ml) or a weak larvicidal agent (LC_{50} > 100 μg/ml). Hence, the oil of *N. cataria* had a strong larvicidal activity which was near to that of thymol and two folds lower than eugenol. The remaining plant oils were moderately active. The insecticidal action of essential oils can be due to their major constituents or to the joint effect of minor and major constituents (Dias and Moraes, 2014). To clarify this point, we performed a principal component analysis (PCA) based on the relative participation of the oil constituents and the larvicidal activity. The PCA suggests that minor and major

Table 2
Concentrations required to kill 50% (LC_{50}) and 95% (LC_{95}) of the *A. aegypti* larvae obtained for the essential oils, thymol, eugenol and chlorpyrifos.

	LC_{50} (μg/ml)[1]	LC_{95} (μg/ml)[1]	Slope ± SD	Chi square (χ^2)
P. harmala oil	101.5 (92.4–109.4)	146.8 (134.5–152.9)	2.5 ± 0.26	10.99
N. cataria oil	47.3 (44.0–51.0)	86.8 (81.3–95.2)	1.83 ± 0.17	11.40
P. amurense oil	72.7 (68.0–78.7)	109.4 (103.0–121.4)	2.37 ± 0.22	12.63
Thymol	37.1 (34.3–40.0)	54.1 (48.2–59.7)	1.91 ± 0.20	10.02
Eugenol	19.8 (19.4–20.9)	35.3 (33.3–39.8)	1.32 ± 0.13	9.64
Chlorpyrifos	2.1 (2.0–2.3)	6.05 (5.7–6.3)	0.91 ± 0.01	3.29

[1] Lower and upper limits of the 95% confidence interval are stated into brackets.

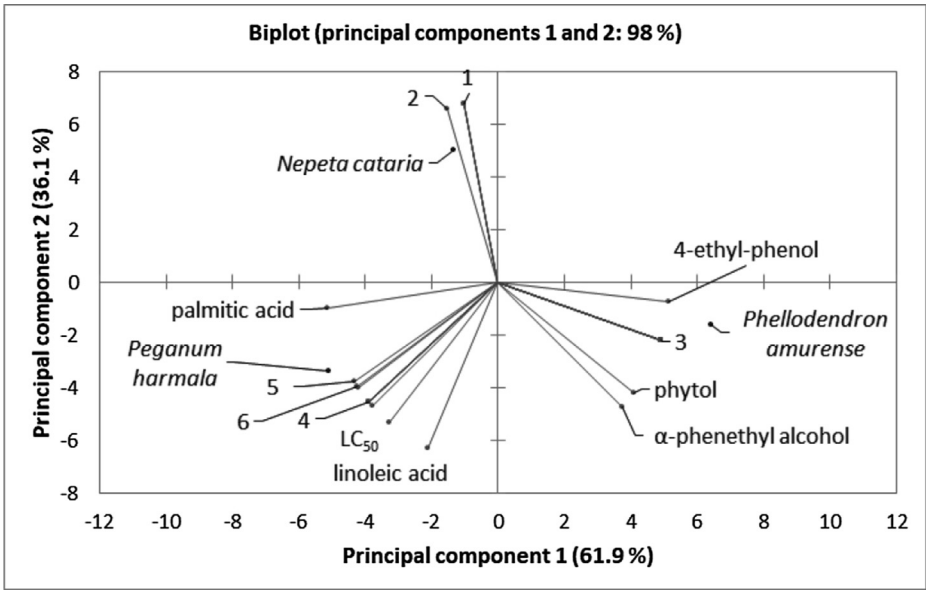

Fig. 1. Bi-plot of the two first principal components computed for the contents of the essential oil constituents and the LC_{50} values recorded for *Aedes aegypti*. 1 = 4aα,7β,7aα-nepetalactone, 4aα,7α,7aβ-nepetalactone, 1,8-cineol, camphor. 2 = thymol. 3 = β-eudesmol, γ-eudesmol, α-eudesmol, caryophyllene oxide, α-bulnesene, *trans*-linalool oxide, eicosane, methyl eugenol, 4-vinylguaiacol, eugenol, *trans*-nerolidol, phenol, benzyl alcohol, 4-methyl phenol. 4 = pyridine, hexadecanoic acid, limonene, *trans*-2-hexenal, dodecanoic acid, decanoic acid, octanoic acid, índole. 5 = 4′-methoxyacetophenone. 6 = linalool, myristic acid.

constituents were involved in the biocidal effect of the *N. cataria* oil. Strong negative correlations were recorded between the LC_{50} values and the contents of 1,8-cineol, camphor and both nepetalactone stereoisomers with correlation coefficients in the range of −0.73 to −0.76 (p = 0.05). Thymol also showed a strong negative correlation (r = −0.64, p = 0.05). Fig. 1 shows these relationships expressed by obtuse angles between the vectors of the mentioned oxygenated monoterpenes and the LC_{50} values obtained for *A. aegypti*. Low and moderate larvicidal activity on *A. aegypti* were reported for 1,8-cineol and camphor when assayed alone with LC_{50} values of 500–1000 μg/ml and 115 μg/ml, respectively (Tyagi et al., 2017). There are no reports about the larvicidal effect of nepetalactone stereoisomers on *A. aegypti*. However, these compounds showed insecticidal and/or repellent activities on several insect species, The 4aα,7α,7aβ-nepetalactone was insecticidal in feeding assays against *Pogonomyrmex* sp. ants (Gkinis et al., 2003). It showed a stronger repellent activity on German cockroach (*Blattella germanica* L.) than equivalent doses either of N,N-diethyl-3-methylbenzamide (DEET) or the 4aα,7α,7aα-stereoisomer which was absent in our *N. cataria* oil (Peterson et al., 2002). Both stereoisomers strongly repelled feeding and oviposition of the stable fly (*Stomoxys calcitrans* L.) (Zhu et al., 2012). Oil samples of *N. cataria* rich in the nepetalactones showed repellent activity on house flies (*Musca domestica* L.), American cockroaches (*Periplaneta americana* L.) and *A. aegypti* sometimes better than that of DEET (Reichert et al., 2019; Schultz et al., 2004).

4. Conclusions

The essential oil from the aerial parts of *N. cataria* showed a strong larvicidal effect which was associated to the contents of five oxygenated monoterpenes (1,8-cineol, camphor, 4aα,7α,7aβ-Nepta lactones, 4aα,7β,7aα-Neptalactones and thymol). Our results indicate that the oil of *N. cataria* deserves to be used as a source of larvicidal agents against *A. aegypti*.

5. Author's contribution

SY, YY, YZ, JQ, YK and DAS designed the study. SY, YY, JQ and YK performed and supervised the plant collections. SY, MB, JY, YY, YZ, JQ and YK performed laboratory investigations. SY, MB, JY and DAS analysed the data. SY and DAS drafted and reviewed the manuscript. All authors read approved the final manuscript.

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Declaration of Competing Interest

We have no conflicts of interest concerning the work reported in this article

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