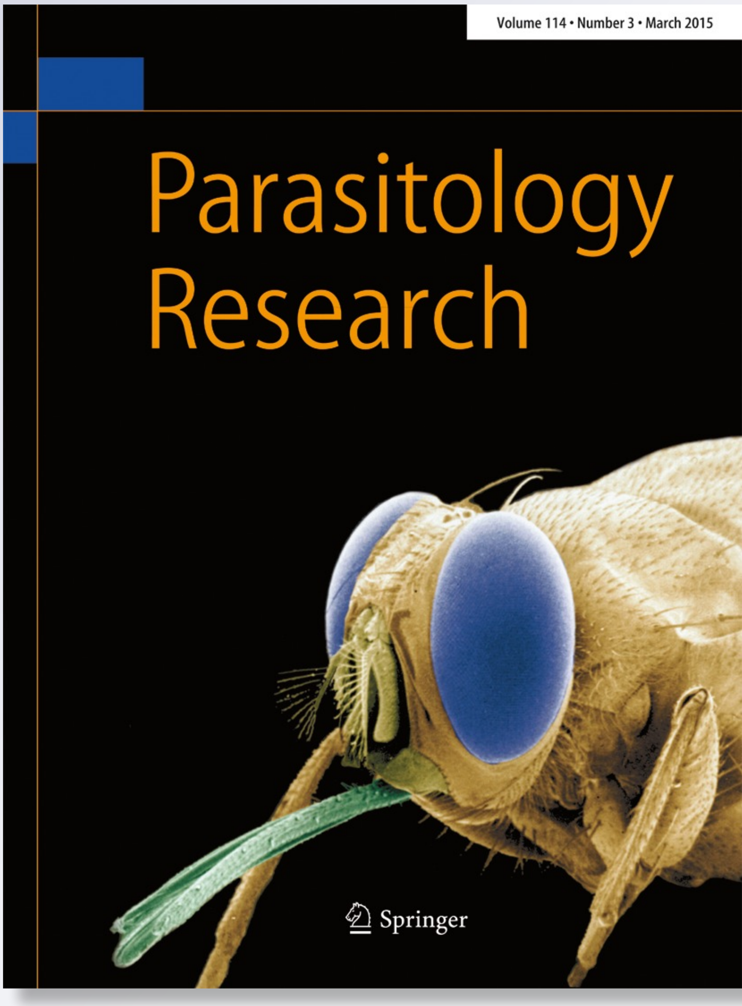
Lethal activity of individual and mixed monoterpenoids of geranium essential Oil on Musca domestica

Anabella Gallardo, María Inés Picollo & Gastón Mougabure-Cueto

Parasitology Research
Founded as Zeitschrift für Parasitenkunde

ISSN 0932-0113
Volume 114
Number 3

Parasitol Res (2015) 114:1229-1232
DOI 10.1007/s00436-015-4315-4



Springer

Parasitol Res (2015) 114:1229–1232
DOI 10.1007/s00436-015-4315-4

SHORT COMMUNICATION

Lethal activity of individual and mixed monoterpenoids of geranium essential Oil on *Musca domestica*

Anabella Gallardo · María Inés Picollo · Gastón Mougabure-Cueto

Received: 28 November 2014 / Accepted: 7 January 2015 / Published online: 22 January 2015

Abstract Plant essential oils and its constituent molecules have been suggested as an alternative to control insect. The contribution of the constituents to the effect of the oil is determined by the interactions occurring between them. Synergistic interactions would improve the insecticide efficacy of the compounds due to the utilization of lower doses. We evaluated the insecticidal activity of geranium *(Geranium maculatum* L.) oil and its major constituents against *Musca domestica* L. and studied the toxic interactions in artificial mixtures of those constituents in the natural ratio. While synergistic interactions were determined in house fly in this study, these were of low intensity evidencing that the effect of each constituent was slightly modified by the other constituents present in the mixtures. The search for synergism between components is a strategy to improve the insecticide activity of natural compounds. The synergism helps to reduce the environmental and toxicological impact due to the reduction of the dose of use.

Keywords Toxic interactions · Monoterpenoids · Synergism · House fly

Introduction

Where conventional insecticides fail as the result of insecticide resistance, investigations continue to focus on the development of insect control alternatives. Plant essential oils are a rich source of bioactive chemicals, as the oil itself and its constituent molecules (e.g., monoterpenoids), and have been suggested as an alternative to control insect pests because of their repellent, ovicidal, adulticidal, and feeding inhibition activities reported against various insect species including house fly (Isman 2000; Palacios et al. 2009; Morey and Khandagle 2012; Kumar et al. 2013; Sinthusiri and Soonwera 2014).

The insecticidal activity of oils is governed by its chemical composition, i.e., the proportion and types of constituent molecules. The contribution of each constituent to the effect of a blend is determined in part by the other constituents present due to pharmacological interactions occurring between them (e.g., synergism). From pest control point of view, synergism allows to improve the insecticide efficacy of natural products due to that a determined effect level can be reach with lower dose (Chou 2006). Synergism between monoterpenoids both natural and artificial mixtures evaluated against diverse pest insects has been reported by different authors (Don Pedro 1996; Hummelbrunner and Isman 2001; Bekele and Hassanali 2001; Jiang et al. 2009; Gallardo et al. 2012).

Thus, the aim of present study was to determine the insecticidal potency of geranium oil and its major constituents against males *Musca domestica* L. (housefly). We also proposed to determine the occurrence of toxic interactions between the major constituents through of evaluation of complex mixtures of them.

Materials and methods

Male *M. domestica* 3–5 days old were used in this study obtained from colony reared in our laboratory in optimal environmental conditions (25±1 °C, 60–75 % RH, and 12:12 L:D photoperiod). Adult flies were fed by placing dry milk, sugar, and water inside 28×28×28-cm boxes. The medium to rear larvae consisted of dried yeast, whole dry milk, agar, and nipagine.

A. Gallardo · M. I. Picollo · G. Mougabure-Cueto (✉)
Centro de Investigaciones de Plagas e Insecticidas (CONICET-CITEDEF), Juan Bautista de la Salle 4397 (B1603ALO), Buenos Aires, Argentina
e-mail: gmougabure@gmail.com

Table 1 Toxic activity by topical application of geranium oil and its major constituents against male *Musca domestica*

Oil/constituents	*n*	X^2	Slope±SE	LD_{50}[a] (95 % CL)
Geranium oil	150	1.493	2.098±0.604	55.34 (33.37–72.44)
Geraniol	150	0.533	5.700±0.956	45.63 (39.35–53.19)
Citronellol	180	0.001	4.893±0.913	59.50 (49.77–68.44)
Linalool	180	0.327	7.319±1.612	106.88 (90.45–118.41)
Citronellyl formate	150	0.012	6.676±1.328	48.06 (41.60–53.35)

[a] Lethal dose 50 expressed in micrograms/insect

Pure compounds (citronellol, geraniol, citronellyl formate, and linalool) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Geranium oil was obtained from Fritzsche Saica (Buenos Aires, Argentina). Analytical grade acetone (Merck, Buenos Aires, Argentina) was used as the carrier.

Acute toxicity of geranium oil, major constituents, and mixtures was determined by topical application on adult males. Citronellol, geraniol, citronellyl formate, and linalool were used as the four major constituents of geranium oil according Gallardo et al (2012). Five mixtures were used: the first contained all major constituents; the remaining four contained all constituents except one. In all cases, constituents were combined in the same ratio as the geranium oil. In all tests, groups of at least 10 insects were treated with 1 μl of acetone solution of the compounds. The flies were anesthetized with CO_2 with a flux of 1–2 l/min. Doses were applied to the abdomen using a repeating topical dispenser attached to a 50-μl syringe (Hamilton Company, Reno). Doses ranged from 2 to 200 μg/insect, and each dose was replicated at least three times. Topical application with acetone alone was made for controls. Treated flies were placed in plastic containers (250 ml) containing a little plastic recipient with cotton moistened with 10 ml of water and covered with a voile. The containers were maintained for 24 h in a growth chamber at rearing conditions. The mortality was recorded at 24 h after topical application. The criterion for mortality was inability to walk and fly.

Mortality data were corrected using Abbott formula (Abbott 1925). Dose-mortality values were subjected to probit regression analysis (Litchfield and Wilcoxon 1949) using POLO-PC Software (LeOra Software 1987). Median lethal dose values (LD_{50}) and confidence intervals were expressed as micrograms per insect. To establish the type of interaction that occurred in each mixture, the combination index (CI) was calculated (Chou 2006). The CI was determined based on the LD_{50} of each constituent and mixture by using CompuSyn (Chou and Martin 2005). A CI of <1, =1, or >1 indicates synergism, an additive effect, and antagonism, respectively (Chou 2006). The grade of each type of interaction (slight, moderate, strong, very strong) was established according Chou (2006).

Results

The toxicological values of individual major components from geranium oil on flies are shown in Table 1. All constituents and the natural oil had similar toxicity, close to 50 μg/insect and overlapping all confidence intervals, except linalool which was less toxic (106.9 μg/insect). The most toxic monoterpenoid constituents were geraniol and citronellyl formate. The toxicological values of mixtures of the major constituents from geranium oil on flies are shown in Table 2. The results showed similar toxicity between all mixtures evaluated and similar to potency of the individual components. The most toxic mixture was Geraniol:Citronellyl formate:Linalool, and the less toxic was the mixture formed by the four major constituents (Geraniol:Citronellol:Citronellyl formate:Linalool). Two mixtures evidenced toxicological interactions as moderate synergism (Geraniol:Citronellyl formate:Linalool and Citronellol:Citronellyl formate:Linalool), and the other mixtures were nearly additives.

Table 2 Toxic activity by topical application of mixtures of major constituents of geranium oil against male *Musca domestica*

Mixture of major constituents[a]	*n*	X^2	Slope±SE	LD $_{50}$[b] (95 % CL)	CI[c]	Type of interaction according to Chou (2006)
Geraniol:Citronellol:Citronellyl formate:Linalool	120	0.067	7.111±1.443	58.23 (51.42–64.97)	1.03	Nearly additive
Geraniol:Citronellol:Citronellyl formate	135	0.095	4.063±0722	56.61 (47.25–68.65)	1.07	Nearly additive
Geraniol:Citronellol:Linalool	110	1.607	3.861±0.621	54.80 (43.95–66.13)	0.93	Nearly additive
Geraniol:Citronellyl formate:Linalool	90	0.292	2.775±0.506	38.50 (28.16–50.69)	0.71	Moderate synergism
Citronellol:Citronellyl formate:Linalool	120	0.876	4.031±0.6	48.52 (41.30–56.74)	0.80	Moderate synergism

[a] Constituents mixed in the same proportion than natural oil

[b] Lethal dose 50 expressed in micrograms/insect

[c] Combination index (CI) of <1, =1, or >1 indicates synergism, an additive effect, and antagonism respectively (Chou 2006)

Discussion

In the present work, we determined the insecticidal activity of geranium oil and its major constituents against males *M. domestica*. Also, we demonstrated the occurrence of toxicological interactions in artificial mixtures of those components in the natural ratio. The toxic interactions determined were moderate synergism and nearly additive.

There are several studies regarding the toxicity of essentials oils or their monoterpenoid constituents on *M. domestica* by topical application. Rice and Coats (1994) evaluated the lethal toxicity of 22 monoterpenoids and obtained LD_{50} values ranging between 33 and >500 μg/insect and compared those of the monoterpenoids studied in the present work. Sukontason et al. (2004) reported LD_{50} of the eucalyptol of 118 μg/insect for male and 177 μg/insect for female which also are in similar order of magnitude of LD_{50} values reported here for other monoterpenoids. However, Tarelli et al (2009), using the same bioassay conditions described in the present study and in the works cited above, reported LD_{50} values of several monoterpenoids shared between studies two or three orders of magnitude more toxic. These authors did not explain the wide difference in toxic potency with the previous reports, but our results agreed with Rice and Coats (1994) and Sukontason et al. (2004).

The contribution of each constituent of a mixture (natural or artificial) on the toxicity of the mixture can depend of the other constituents present in the mixture as consequence of toxicological interactions between them. While interactions were determined in house fly in this study, these were of low intensity evidencing that there was not a constituent with major contributions to the toxicity of the mixtures and that the contribution of each constituent was just gently modified by the other constituents present in the mixtures. In contrast, in a similar study carried out on female head lice, Gallardo et al. (2012) showed that the citronellol was the major contributor to geranium oil toxicity and demonstrated synergism in all mixtures formed by the same major constituents of the oil. Thus, the toxicological differences between insect species are evidenced not only in the toxic potency of the constituents or mixtures but also in the type of toxic interactions that occur between individual monoterpenoids.

Although conventional pesticides are more effective due its higher toxicity, botanical oils and its constituents may have a role in the development of insecticides especially in areas where resistance renders commercial products ineffective. In this context, the search for synergism between components of essential oils is a promising strategy to improve the insecticide activity of natural compounds. The synergistic mixtures allow reaching a determined level of effect using lower dose of its constituents than using the constituents isolated. Thus, the synergism also helps to reduce the environmental and toxicological impact of the compounds due to the reduction of the dose of use.

Acknowledgements We thank the authorities of the elementary schools where head lice were collected. This investigation received financial support from Agencia Nacional de Promoción Científica y Tecnológica (Argentina), Laboratorio Elea (Argentina), and Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina).

References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265–267

Bekele J, Hassanali A (2001) Blend effects in the toxicity of the essential oil constituents of *Ocimum kilimandsharicum* and *Ocimum kenyense* (Labiatae) on two post-harvest insect pests. Phytochem 57:385–391

Chou T-C (2006) Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev 58:621–681

Chou T-C, Martin N (2005) CompuSyn for drug combinations: PC Software and user's guide: a computer program for quantitation of synergism and antagonism in drug combinations, and the determination of IC_{50} and ED_{50} and LD_{50} values. ComboSyn Paramus, NJ

Don-Pedro KN (1996) Investigation of single and joint fumigant insecticidal action of citruspeel oil components. Pestic Sci 46:79–84

Gallardo A, Picollo MI, González-Audino P, Mougabure-Cueto G (2012) Insecticidal activity of individual and mixed monoterpenoids of geranium essential oil against *Pediculus humanus capitis* (Phthiraptera: Pediculidae). J Med Entomol 49:332–335. doi: 10.1603/ME11142

Hummelbrunner LA, Isman MB (2001) Acute, sublethal, antifeedant, and synergistic effects of monoterpenoids essential oil compounds on the tobacco cutworm, *Spodoptera litura*. J Agric Food Chem 49: 715–720

Isman M (2000) Plant essential oils for pest and disease management. Crop Prot 19:603–608

Jiang Z, Akhtar Y, Bradbury R, Zhang X, Isman MB (2009) Comparative toxicity of essential oils of *Litsea pungens* and *Litsea cubeba* and blends of their major constituents against the cabbage looper, *Trichoplusia ni*. J Agric Food Chem 10:4833–4837

Kumar P, Mishra S, Malik A, Satya S (2013) Housefly (*Musca domestica* L.) control potential of *Cymbopogon citratus* Stapf. (Poales: Poaceae) essential oil and monoterpenes (citral and 1.8 cineole). Parasitol Res 112:69–76

LeOra Software (1987) POLO-PC: a user´s guide to probit or logit analysis. LeOra Software, Berkeley, CA

Lichfield JT, Wilcoxon FJ (1949) A simplifed method of evaluating dose-effect experiments. J Exp Therap 96:99–100

Morey RA, Khandagle AJ (2012) Bioefficacy of essential oils of medicinal plants against housefly *Musca domestica* L. Parasitol Res 111: 1799–1805

Palacios SM, Bertoni A, Rossi Y, Santander R, Urzúa A (2009) Insecticidal activity of essentials oils from native medicinal plants of Central Argentina against the house fly *Musca domestica* (L.). Parasitol Res 106:207–212

Rice PJ, Coats JR (1994) Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern corn rootworm (Coleoptera: Chrysomelidae). J Econ Entomol 87:1172–1179

Sinthusiri J, Soonwera M (2014) Oviposition deterrent and ovicidal activities of seven herbal essential oils against female adults of housefly, *Musca domestica* L. Parasitol Res 113:3015–3022

Sukontason KL, Boochu N, Sukontason K, Choochote W (2004) Effects of eucalyptol on house fly (Diptera: Muscidae) and blow fly (Diptera: Calliphoridae). Rev Inst Med Trop Sao Pablo 46:97–101

Tarelli G, Zerba EN, Alzogaray R (2009) Topical application pf essential oils and monoterpenoids on *Musca domestica* (Diptera: Muscidae). J Econ Entomol 102:1383–1388