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Acaricidal and larvicidal activity of *Piper marginatum* and *Piper tuberculatum* essential oils from Ecuador

Actividad acaricida y larvicida de los aceites esenciales de *Piper marginatum* y *Piper tuberculatum* de Ecuador

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Larvae of *A*. *aegypty* and *R*. *microplus* after treatment with essential oils of *P*. *marginatum* (top) and *P*. *tuberculatum* (bottom).
Photo: S.M. Moncayo-Baño

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ABSTRACT

Piper marginatum and *Piper tuberculatum* are species used by local indigenous, people who live especially in eastern Ecuador, for their insecticidal properties. This study aimed to determine the acaricidal and larvicidal activity of the essential oil of these species. The essential oils were obtained by hydrodistillation. The acaricidal activity was evaluated with the contact toxicity method against larvae of the tick *Rhipicephalus microplus*. Larvicidal activity was determined against *Aedes aegypti* mosquito larvae following WHO recommended guidelines. The essential oils of *P. marginatum* and *P. tuberculatum* showed an acaricidal potential with LC_{50} of 0.90 and 0.73 µL mL^{-1}, respectively. In addition, excellent larvicidal activity was obtained with LC_{50} of 11.87 µL mL^{-1} (*P. marginatum*) and 8.42 µL mL^{-1} (*P. tuberculatum*) at 48 hours of evaluation. This is the first report of the acaricidal activity of *P. marginatum* and *P. tuberculatum* essential oils against *Rhipicephalus microplus* tick larvae. The results indicate that essential oils could be a natural alternative for the controlling these pests and would justify their ethnobotanical applications as a lice and tick repellent.

Additional key words: *Piper*; larvae*; Rhipicephalus microplus; Aedes aegypti;* ethnobotanical.

RESUMEN

Piper marginatum y *Piper tuberculatum* son especies empleadas por indígenas locales, personas que habitan especialmente la zona Oriental de Ecuador, por sus propiedades insecticidas. Este estudio tuvo como objetivo determinar la actividad acaricida y larvicida del aceite esencial de estas especies. Los aceites esenciales se obtuvieron por hidrodestilación. La actividad acaricida se evaluó con el método de toxicidad por contacto frente a larvas de la garrapata *Rhipicephalus microplus*. La actividad larvicida se determinó frente a larvas del mosquito *Aedes aegypti* siguiendo los lineamientos recomendados por la OMS. Los aceites esenciales de *P. marginatum* y

P. tuberculatum mostraron un potencial acaricida con CL_{50} de 0.90 y 0.73 µL mL^{-1}, respectivamente. Además, se obtuvo una excelente actividad larvicida con CL_{50} de 11.87 µL mL^{-1} (*P. marginatum*) y 8.42 µL mL^{-1} (*P. tuberculatum*) a las 48 horas de evaluación. Este es el primer reporte de la actividad acaricida de los aceites esenciales de *P. marginatum* y *P. tuberculatum* frente a las larvas de la garrapata *Rhipicephalus microplus*. Los resultados indican que los aceites esenciales podrían ser una alternativa natural para el control de estas plagas y justificaría sus aplicaciones etnobotánicas como repelente de piojos y garrapatas.

Palabras clave adicionales: *Piper*; larvas; *Rhipicephalus microplus; Aedes aegypti*; etnobotánica.

INTRODUCTION

The most important tick in livestock worldwide is *Rhipicephalus (Boophilus) microplus*. This hematophagous ectoparasite is one of the main obstacles to the productivity of the livestock industry in areas where this species is endemic (Nicaretta *et al*., 2021). They a cause decrease in cattle weight gain, damage to hides, a decrease in meat and milk production, myiasis and transmission of zoonotic diseases (Polanco and Ríos, 2016). Due to its importance, it is the most used species in acaricide trials (Adenubi *et al*., 2018). *Aedes aegypti* are vectors of arboviral pathogens that cause hundreds of millions of cases annually of Zika, dengue fever, yellow fever, and chikungunya. They thrive in urbanized areas where they are in close contact with people, making them an exceptionally successful vector. *A. aegypti* is extremely common in areas that lack piped water systems and rely heavily on stored water for breeding sites (Matthews, 2019; Zettel and Kaufman, 2019).

In preventing of vector-borne diseases, there is a trend to develop alternative methods based on natural products to replace chemical pesticides and delay the development of resistance, a problem that causes millions of dollars in losses (Selles *et al*., 2021). Essential oils are metabolites formed by a complex mixture of several chemical compounds mainly terpenes and phenylpropanoids, which give plants their characteristic aroma and possess a wide range of biological properties, including antimicrobial, antifungal and insecticidal activities (De Souza *et al*., 2023). Studies conducted on the essential oils of *Piper* species (Piperaceae) are characterized by their great potential for pest control and the low environmental impact observed during the

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process (Pereira *et al*., 2021). Their efficacy against different species of mosquitoes and mites at low concentrations has been reported (Huong *et al.,* 2019; Araújo *et al.,* 2020).

Piper marginatum Jacq. and *Piper tuberculatum* Jacq. are native neotropical species, that stand out for their pharmacological potential, being widely used in folk medicine for their healing properties (Salehi *et al*., 2019). *P. marginatum* is known in Ecuador as cordoncillo blanco, Santa María and "Anotede" in Tsa'fiki language (De la Torre, 2008). Its essential oils have shown antibacterial, antioxidant, antiparasitic and larvicidal activities (Ayres *et al*., 2021). *P. tuberculatum* is used in Ecuador to combat pediculosis problems (De la Torre *et al*., 2008). Previous studies have shown that its essential oils have antimicrobial, insecticidal and antiprotozoal effects (Da Silva *et al*., 2023).

This study reports the acaricidal and larvicidal activity of the essential oils of *P. marginatum* and *P. tuberculatum* against larvae of the tick *R. microplus* and the mosquito *A. aegypti*, species that cause serious damage to human and animal health and serious economic losses.

MATERIALS AND METHODS

Plant materials

Leaves, stems, and inflorescences of *P. marginatum* and *P. tuberculatum* were collected in Cumanda, Chimborazo province (2°12'12" S, 79°06'36" W) and on the El Triunfo - Bucay road, Guayas province (2°18'9" S, 79°14'16" W) in western Ecuador, respectively. The identification of the botanical species was carried out in the GUAY Herbarium of the Faculty of Natural Sciences of the University of Guayaquil, Ecuador, where the vouchers of the specimens were deposited under the codes MER06 (*P. marginatum*) and MER07 (*P. tuberculatum*).

Hydrodistillation

The leaves, stems, and inflorescences of the species were subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus. The oils, once obtained, were meticulously dried over anhydrous sodium sulfate and then stored in sealed vials. These vials were then placed in a controlled environment at 4°C in the dark, ensuring the oils' stability and integrity until they were ready for analysis and testing.

Acaricidal activity

It was performed using the contact toxicity technique described by Tabari *et al*. (2020) with minor modifications. Larvae between 14 and 21 d old of the tick *R. microplus* obtained from gravid females collected from infested cattle were used. The tick species was identified by Prof. Antonio Ascencão of the Laboratory of Biodiversity of Arthropods, Department of Biology, Faculty of Sciences, University of Los Andes, Venezuela. Serial of essential oil dilutions were prepared from 20 to 0.3 µL mL^{-1}, in 2% Tween® 80 solution. A Tween® 80 solution was used as a negative control and a 20 µL mL^{-1} cypermethrin solution dissolved in Tween® 80 (2%), prepared from the commercial product CIPER-VEEX® (10% cypermethrin), was used as a positive control. Whatman® N°1 filter paper discs were impregnated with 1 mL of the test solutions. Tick larvae were transferred between two of the impregnated paper disks, placed inside a Petri dish. The plates were sealed and stored in a glass chamber at 27±2°C and 85±2% humidity. Assays were performed in duplicate. Mortality records were made after 48 h under a stereomicroscope.

Larvicidal activity

It was performed following the guidelines of the World Health Organization (WHO, 1981) against third and fourth instar larvae of wild *A. aegypti*, collected at the Faculty of Natural Sciences of the University of Guayaquil. The larvae were identified by the author with the help of taxonomic guides. An aliquot of essential oils was solubilized in absolute ethanol until a stock solution was obtained, with which test solutions were prepared in serial dilutions of 1,000 to 3 µL mL^{-1}. The final volume of the solutions was 50 mL. A total of 10 larvae were exposed to the test solutions. The negative control consisted of a 2% ethanol solution; as a positive control, a solution of the organophosphate insecticide malathion (1 µL mL^{-1}) was used. Each concentration was evaluated in triplicate. The assays were stored at 25±2ºC and relative humidity of 70±2%. Larval mortality readings were taken at 24 and 48 h.

Statistical analyses

The concentrations producing 50 and 95% mortality in the mite and larvae population (LC_{50}) and (LC_{95}) and their respective confidence limits (95 %) were calculated by Probit analysis. To establish statistically significant differences between the assays, a one-way ANOVA analysis of variance was performed using the Fisher (LSD) method. Values of P<0.05 were considered significant. All the statistical analyses were carried out using Statgraphics Centurion XVI statistical software.

RESULTS AND DISCUSSION

Acaricidal activity

The mortality percentages of *R. microplus* larvae after 48 h of exposure to the essential oils of *P. marginatum* and *P. tuberculatum* are shown in tables 1 and 2. No mortality was reported in the negative control, while mortality with the positive control was 100%. The results indicate that the essential oil of *P. tuberculatum* was the most toxic, causing 100% mortality of *R. microplus* larvae at a concentration of 1 µL mL^{-1}. ANOVA comparison analysis found statistically significant differences between the lethal concentrations of the essential oils at 95 % significance level.

Table 1. Mortality percentage of *R. microplus* caused by different concentrations of *Piper marginatum* essential oil.

Concentration (µL mL^{-1})	0.6	1.2	2.5	5.0	10.0	20.0
Mortality	11±2	86±1	92±3	100±0	100±0	100±0

Mean of *n*=3±standard deviation.

Table 2. Mortality percentage of larvae of *R. microplus* caused by different concentrations of *Piper tuberculatum* essential oil.

Concentration (µL mL^{-1})	0.3	0.6	0.8	1.0	2.5
Mortality	6±1	23±2	49±1	100±0	100±0

Mean of *n*=3±standard deviation.

Table 3 summarizes the LC_{50} and LC_{90} values of *P. marginatum* and *P. tuberculatum* oils against *R. microplus* tick larvae. The most potent oil was that of *P. tuberculatum*, with a LC_{50} of 0.73 µL mL^{-1} while the oil of *P. marginatum* showed an LC_{50} of 0.90 µL mL^{-1}.

Table 3. Lethal concentrations of the essential oils of *Piper* species on larvae of *R. microplus*.

Essential oil	LC_{50}	LC_{95}
P. marginatum	0.90 (0.75-1.02)	2.10 (1.86-2.47)
P. tuberculatum	0.73 (0.71-0.75)	1.06 (1.02-1.11)

(95 % CI) = 95 % confidence interval

This groundbreaking report unveils the acaricidal activity of essential oils from *Piper* species against *R. microplus* larvae, a discovery of utmost significance as larvae are pivotal in tick control systems. The authors' findings suggest that essential oils from plants of the genus *Piper*, rich in phenylpropanoids and hydrocarbonated sesquiterpenes as in this study, exhibit potent insecticidal and acaricidal activity (Da Silva *et al*., 2017).

The practical implications of this research are evident in the evaluation of the essential oil of *P. marginatum* and *P. tuberculatum* against the mite *Tetranychus urticae*, a crop pest from the family Tetranychidae. The obtained LC_{50} values of 0.90 µL mL^{-1} and 0.50 µL mL^{-1}, respectively (Ribeiro *et al*., 2016; Araújo *et al*., 2020) underscore the potential of these essential oils in pest control.

According to Selles *et al*. (2021), the acaricidal activity of essential oils is related to their hydrophobic nature, which is responsible for several mechanical effects, causing death by water stress or asphyxia due to alteration of cuticular waxes and blockage of respiratory stigmas. Essential oils have been shown to act on the motor function of *R. microplus* larvae, increasing catecholamine levels within the central nervous system (Salman *et al*., 2020) and inhibit the action of acetylcholinesterase in this tick species (Cardoso *et al*., 2020).

The acaricidal capacity observed in the extracts would justify the ethnobotanical use of *P. tuberculatum* in Ecuador as a lice and tick repellent (De la Torre *et al*., 2008).

Larvicidal activity

The concentrations of the essential oils and their respective percentages of mortality after 24 and 48 h of exposure are shown in table 4 and 5. *A. aegypti* larvae's mortality percentage was 100 % from the concentration of 50 µL mL^{-1} in both oils evaluated. No mortality was reported in the negative control, while mortality with the positive control evaluated was 100 %.

Table 4. Mortality percentage of *Aedes aegypti* larvae caused by different concentrations of *Piper marginatum* essential oil (µL mL^{-1}).

Exposure time (h)	3	6	12	16	20	25	50-250
24	10±0	27±6	30±0	53±6	73±6	83±6	100±0
48	17±6	30±6	43±6	67±6	87±15	90±0	100±0

Mean of *n*=3±standard deviation.

Table 5. **Mortality percentage of *Aedes aegypti* larvae caused by different concentrations of *Piper tuberculatum* essential oil (µL mL^{-1}).**

Exposure time (h)	3	6	12	25	30	40-250
24	17±6	30±10	33±6	60±0	70±10	100±0
48	30±10	57±6	60±10	70±0	80±10	100±0

Mean of *n*=3±standard deviation.

The lethal concentrations of the essential oils are shown in the table 6. *P. tuberculatum* obtained the highest activity with LC_{50} of 8.42 ppm and LC_{95} of 51.38 ppm while *P. marginatum* obtained LC_{50} of 11.87 ppm and LC_{95} of 28.29 ppm at 48 h of exposure. ANOVA comparison analysis found statistically significant differences between the lethal concentrations of the essential oils at 95 % significance level.

Table 6. **Lethal concentrations (LC) of the essential oils of *Pirper marginatum* and *P. tuberculatum* species on 3°- 4° instar larvae of the mosquito *Aedes aegypti*.**

Exposure time (h)	LC	*P. marginatum*	*P. tuberculatum*
24	LC_{50} (CI)	14.65 (11.90-17.84)	19.70 (15.43-25.53)
	LC_{95} (CI)	32.34 (26.95-42.53)	52.69 (41.40-78.73)
48	LC_{50} (CI)	11.87 (9.21-14.61)	8.42 (4.25-13.08)
	LC_{95} (CI)	28.29 (23.63-36.83)	51.38 (37.76-94.06)

(95% CI) = 95% confidence interval.

The volatile compounds of *Piper* species are characterized by their great potential for pest control and the low environmental impact observed during the process (Pereira *et al*., 2021). Previous larvicidal evaluations have been performed on the oils of *P. marginatum* and *P. tuberculatum* (Lavor *et al*., 2012; Pereira *et al*., 2021), which obtained LC_{50} of 39 ppm and 106 ppm, respectively. Essential oils from Ecuadorian species showed higher larvicidal activity, probably related to their different chemical composition and synergistic interactions between their compounds. The plant extracts utilized in the present study were previously characterized to identify their phytochemical composition by our research team, identifying compounds such as sesquiterpenes curzerene (22.44%) and atractylone (18.15%) in *P. marginatum*, while in *P. tuberculatum*, the phenylpropanoid dilapiol (49.15%) was identified (Moncayo *et al*., 2021). Species with essential oils composed mostly of these compounds have shown insecticidal properties against adults and larvae of *A. aegypti* and *Culex quinquefasciatus* mosquitoes (Benelli *et al*., 2017; Hung *et al*., 2020; Pereira *et al.,* 2021). Dilapiol, a potent inhibitor of cytochrome activity (P450 and CYP34A), reduces the ability to excrete xenobiotics causing the insect's death by accumulation of toxic substances in the digestive tract (Santos *et al*., 2021). In addition, it possesses a structure capable of amplifying the effects of other chemicals (Durofil *et al*., 2021).

CONCLUSION

The present study demonstrated that the essential oils of *P*. *marginatum* and *P*. *tuberculatum* possess high acaricidal (*R*. *microplus*) and larvicidal (*A*. *aegypti*) activity and could be used as a natural alternative for the biocontrol of these dangerous disease vectors. This is the first report on the activity of *P*. *marginatum* and *P*. *tuberculatum* essential oils against *R*. *microplus* tick larvae.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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