Short Communication

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Larvicidal activity of oils, fatty acids, and methyl esters from ripe and unripe fruit of *Solanum lycocarpum* (Solanaceae) against the vector *Culex quinquefasciatus* (Diptera: Culicidae)

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ABSTRACT

Introduction: The larvicidal activity of oils, fatty acids, and methyl esters of *Solanum lycocarpum* fruit against *Culex quinquefasciatus* is unknown. **Methods:** The larvicidal activity of samples of ripe and unripe fruit from *S. lycocarpum* was evaluated against third and fourth instar larvae of *C. quinquefasciatus*. **Results:** The oils, fatty acids, and methyl esters of *S. lycocarpum* showed the greatest larvicidal effect (57.1-95.0%) at a concentration of 100mg/L (LC_{50} values between 0.70 and 27.54mg/L). **Conclusions:** *Solanum lycocarpum* fruit may be a good source of new natural products with larvicidal activity.

Keywords: Bioassay. Mosquito. Pesticide.

Synthetic insecticides are used to control mosquito vectors of diseases in several parts of the world. However, resistance to synthetic insecticides has recently become problematic in vector control programs. Thus is important the development of new products, with the capacity to prevent or minimize the resistance, to combat insects(1). Biological products represent alternative approaches for preventing the development of resistance in mosquitoes.

Plants contain bioactive compounds with insecticidal properties that could be suitable for mosquito control applications(2). Several species of the genus *Solanum* have demonstrated larvicidal and pupicidal activities against the mosquito *Culex quinquefasciatus*(3) (4) (5). *Solanum lycocarpum* A. St. Hil. (Solanaceae) is popularly known as the *fruit of the wolf* and is widely distributed in the Brazilian Cerrado(6). *Solanum lycocarpum* is commonly used in traditional medicine as a sedative and a treatment for epilepsy, asthma, diabetes, obesity, abdominal pain, renal pain, and high cholesterol levels(7). Despite the widespread medicinal usage of *S. lycocarpum* and the reported insecticidal properties of other *Solanum* species, no studies have been conducted on the insecticidal activity of oils, fatty acids, and methyl esters obtained from the ripe and unripe fruit of *S. lycocarpum*.

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Fruit of *S. lycocarpum* A. St. Hil. were collected in São Sebastião do Oeste, Minas Gerais, Brazil in August 2011. The plant material was identified by Dr. Alexandre Salino. A voucher specimen (BHCB 159397) was deposited at the *Instituto de Ciências Biológicas Herbarium, Universidade Federal de Minas Gerais*, Belo Horizonte, Minas Gerais, Brazil. Samples of the dried and powdered unripe (170.01g) and ripe (250.58g) fruit were subjected to oil extraction using a Soxhlet extractor with petroleum ether as the solvent (Vetec®, São Paulo, Brazil; 700mL, 6h). The extracted oils were concentrated in a rotary evaporator at 50°C under reduced pressure to produce 26.95g of oil of unripe fruit (OUF) and 29.09g of oil of ripe fruit (ORF).

Fatty acids and methyl esters were isolated from *S. lycocarpum* via transesterification(8). OUF and ORF (2g each) were refluxed with 1.0 mol/L methanolic NaOH solution for 30 min and extracted with ethyl ether. The aqueous phases were acidified with 1.0 mol/L HCl solution and extracted with ethyl ether to obtain fatty acids, which were dissolved in hexane and refluxed with H_2SO_4 methanolic solution (2% v/v) for 1h to obtain methyl esters. The resulting samples were concentrated in a rotary evaporator at 35°C under reduced pressure to obtain 0.34g of fatty acids of unripe fruit (FAUF), 0.29g of fatty acids of ripe fruit (FARF), 1.27g of methyl esters of unripe fruit (MEUF), and 0.94g of methyl esters of ripe fruit (MERF).

Gas chromatography-mass spectrometry (GC-MS) of oils was performed on a Shimadzu QP5050A apparatus with electron ionization at 1.2kV and helium as the carrier gas. A DB-5 [(5% phenyl)-methylpolysiloxane] column (30m × 0.25mm, 0.25mm i.d.) was used. The temperature was initially held at 80°C and increased to 300°C in increments of 5°C/min.

The injection and detector temperature was 250°C. The split ratio was 1/10. The oven flow was 2mL/min and the mass range was 50-500*m/z*. Methyl esters were identified by comparison of their retention times with those of standards and by using the *National Institute of Standards and Technology* (NIST) 2.0 database.

Culex quinquefasciatus larvae were obtained from their biotope as described by Gerberg[9]. Third and fourth instar larvae of *C. quinquefasciatus* were exposed to different concentrations (0.0011, 0.33, and 100mg/L; dissolved in 1% dimethyl sulfoxide) of the oils, fatty acids, and methyl esters of *S. lycocarpum* until the emergence of adults to determine the optimal sub-lethal concentration of the extracted compounds. For each test sample, larvae were divided into test and control groups consisting of 60 specimens each, with 3 replicates for each treatment. The control group was exposed to 1% dimethyl sulfoxide in water. The temperature was maintained at 26 ± 1°C throughout all of the tests. The larvicidal bioassay was performed according to the World Health Organization standard protocol[10]. Larvae were exposed to the test solutions and mortality was recorded every 24h over a period of 144h. Three replicates of each treatment were performed. All tested larvae were of the first generation. Larvae were considered dead when they did not respond to the stimulus or rise to the surface of the solution. LC_{50} and LC_{90} values were calculated by Probit regression (GW Basic 3.22). Differences between the treatment groups were analyzed by Tukey's test, with results of $p < 0.05$ considered statistically significant.

In this study, oils obtained from the fruit of *S. lycocarpum* were analyzed by GC-MS as methyl esters. In *S. lycocarpum* fruit, unsaturated fatty acids oleic acid and linoleic acid were detected. Palmitic acid was the most abundant saturated fatty acid, followed by stearic acid (**Table 1**). Unripe *S. lycocarpum* fruit exhibited high linoleic acid content (75.5%). Palmitic acid and oleic acid were the predominant fatty acids in ripe *S. lycocarpum* fruit.

The larval mortality rates of each group are shown in **Figure 1**. The larvicidal effects in each experiment were dependent on the particular test solution and concentration that was utilized. The mortality rate was directly proportional to the concentration of oils, fatty acids, or methyl esters to which the larvae were exposed **(Figure 1)**. FAUF, MERF, OUF, and FARF were most effective at a concentration of 100g/L, with mortality rates of 95%, 93.3%, 90.8%, and 88.9%, respectively, which were significantly different than the rates produced by the other treatments ($p < 0.05$). The lowest tested sample concentrations (0.0011 and 0.33mg/L) showed the lowest mortality rates (**Figure 1**). There was a complete absence of larval mortality in the dimethyl sulfoxide control group

The LC_{50} and LC_{90} values for the tested oils, fatty acids, and methyl esters as estimated by the LC_{50}-LC_{90} regression equation, as well as the results of chi-square tests, are presented in **Table 2**. The tested oils, fatty acids, and methyl esters had larvicidal effects against *C. quinquefasciatus,* with LC_{50} values between 0.70 and 27.54mg/L, demonstrating more potent larvicidal activity than that of other plant species of the *Solanum* genus. Ethanolic extracts of the leaves of *Solanum xanthocarpum* showed larvicidal activity against third and fourth instar larvae of *C. quinquefasciatus*[3], with LC_{50} values of 271.12 and 377.40mg/L, respectively. Sakthivadivel and Daniel[4] demonstrated the larvicidal effect of the ethanolic extract of leaves of *Solanum trilobatum* on fourth instar larvae of *C. quinquefasciatus*, with an LC_{50} value >200mg/L. In a recent study[5], methanolic extract and extract fractions from unripe *S. lycocarpum* fruit showed larvicidal effects against *C. quinquefasciatus,* with LC_{50} values of 75.13-207.05mg/L.

In this study, OUF, FAUF, FARF, and MERF showed the lowest LC_{90} values (139.47, 93.03, 147.02, and 125.13mg/L, respectively) and were thus the most toxic of the fractions against *C. quinquefasciatus*. Ethanolic extracts of the leaves of *S. xanthocarpum* also showed activity against third and fourth instar larvae of *C. quinquefasciatus*[4], with LC_{90} values of 1,011.89 and 1,058.85mg/L, respectively. Changbunjong et al.[11] showed that ethanolic extract of unripe *S. xanthocarpum* fruit had larvicidal activity against *C. quinquefasciatus,* with LC_{50} and LC_{90} values of 573.20 and 1,066.93mg/L, respectively, indicating that the extract was less toxic than that tested in this study.

TABLE 1 - Fatty acid composition and oil content of *Solanum lycocarpum* fruit.

Type of fatty acid	Unripe fruit (%)	Ripe fruit (%)
$C_{13:0}$ (tridecanoic acid)	0.9	3.1
$C_{16:0}$ (hexadecanoic acid or palmitic acid)	6.7	41.5
$C_{18:0}$ (octadecanoic acid or stearic acid)	3.3	18.4
$C_{18:1}$ (9-octacedenoic acid or oleic acid)	13.1	26.9
$C_{18:2}$ (9,12-octadecadienoic acid or linoleic acid)	75.5	6.3
$C_{22:0}$ (docosanoic acid or behenic acid)	Tr	1.4
$C_{24:0}$ (tetracosanoic acid)	0.4	2.3
Total	**99.9**	**99.9**

Tr: trace.

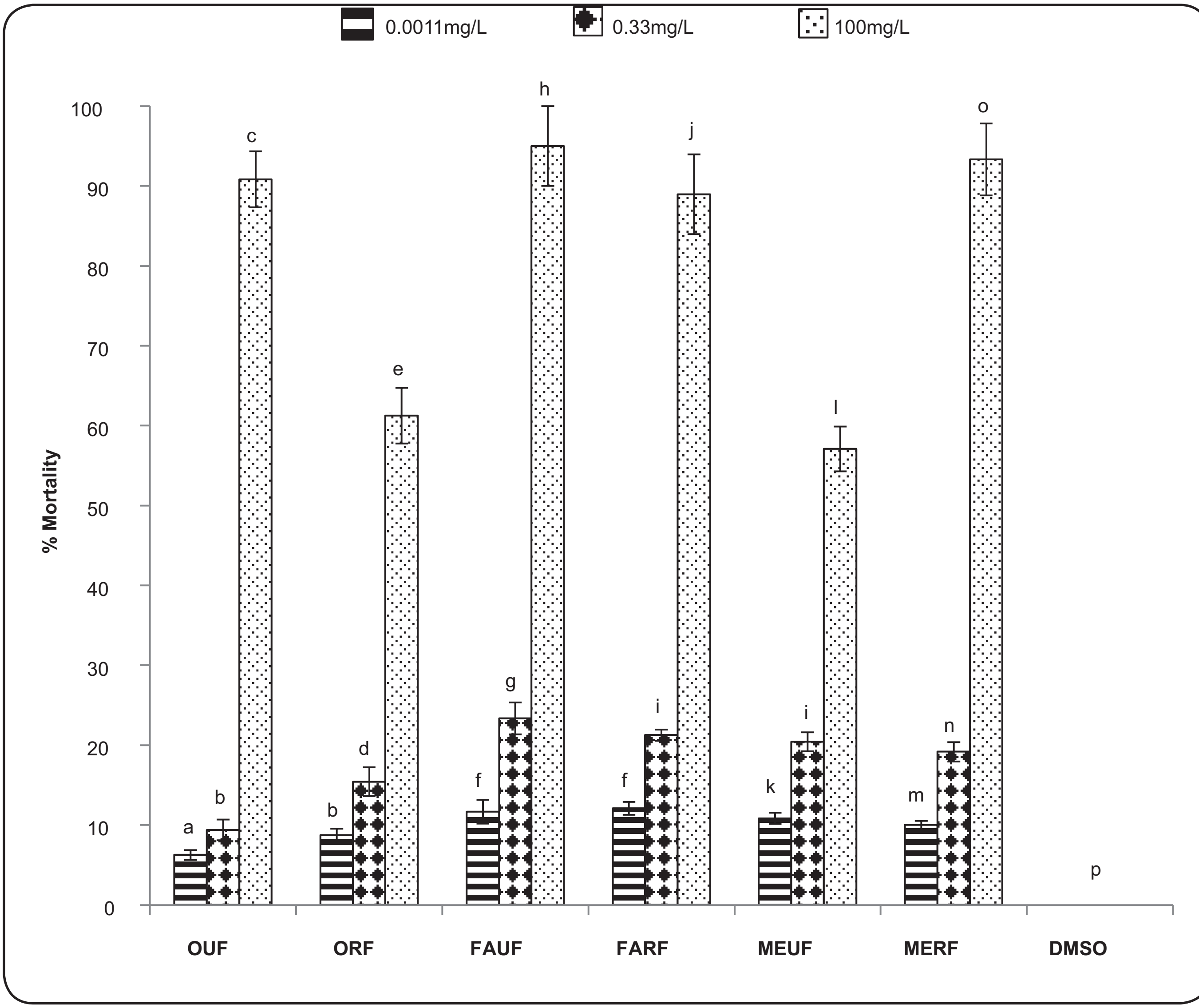

FIGURE 1 - Larval mortality rate of *Culex quinquefasciatus* after exposure to different concentrations of the oils, fatty acids, and methyl esters of unripe and ripe *Solanum lycocarpum* fruit. Different letters in each column indicate significant differences ($p < 0.05$) based on Tukey's test. **OUF:** oil of unripe fruit; **ORF:** oil of ripe fruit; **FAUF:** fatty acids of unripe fruit; **FARF:** fatty acids of ripe fruit; **MEUF:** methyl esters of unripe fruit; **MERF:** methyl esters of ripe fruit; **DMSO:** dimethyl sulfoxide (control treatment).

Kannathasan et al.[12] evaluated the larvicidal activity of fatty acid methyl esters (FAMEs) obtained from 3 species of *Vitex* against *C. quinquefasciatus* larvae and reported LC_{50} values ranging from 9.25 to 18.64mg/L. In this study, MEUF and MERF showed the lowest LC_{50} values (3.72 and 2.16mg/L, respectively) of the test treatments, indicating more potent larvicidal activity than FAMEs isolated from *Vitex* species. The difference in the potency of FAMEs isolated from *Vitex* species and methyl esters from *S. lycocarpum* may be attributed to differences in methyl ester composition; palmitic and linolenic acid are predominant in *Vitex* species.

Some experiments show that biological activity against mosquitoes is mainly due to the presence of long-chain unsaturated fatty acids, rather than of saturated or methylated forms[13] [14], corroborating the results of this study, in which fatty acids (FARF and FAUF) had the lowest LC_{50} values and were thus the most potent larvicides. Nevertheless, oils (ORF and OUF) and methyl esters (MERF and MEUF) from ripe and unripe *S. lycocarpum* fruit also showed larvicidal activity against *C. quinquefasciatus*. These results form the basis for additional studies aimed at evaluating the potential of oils, fatty acids, and methyl esters isolated from ripe and unripe *S. lycocarpum* fruit as natural, plant-based larvicide sources.

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TABLE 2 - Lethal concentrations of oils, fatty acids, and methyl esters of unripe and ripe of *Solanum lycocarpum* fruit in *Culex quinquefasciatus*.

Sample	LC_{50} (95% CI) mg/L	LC_{90} (95% CI) mg/L	Regression equation	χ^2	p-value
OUF	2.99 (1.94-4.63)	139.47 (35.77-543.82)	y = 4.778*x* + 0.538	5.36	0.25
ORF	27.54 (13.11-57.88)	1,362.42 (126.54-1,467.26)	y = 4.332x + 0.425	8.33	0.21
FAUF	0.70 (0.44-1.12)	93.03 (22.61-382.85)	y = 5.151x + 0.539	13.33	0.14
FARF	1.33 (0.82-2.17)	147.20 (26.85-806.99)	y = 4.942x + 0.538	4.29	0.66
MEUF	3.72 (0.42-3.92)	2,534.55 (156,00-4,178,05)	y = 4.863x + 0.511	8.29	0.08
MERF	2.16 (1.35-3.45)	125.13 (30.25-517.46)	y = 4.942x + 0.658	13.18	0.38

LC_{50}: median lethal concentration; **95% CI:** 95% confidence interval; **LC_{90}:** 90% lethal concentration; **χ^2:** chi-square. **OUF:** oil of unripe fruit; **ORF:** oil of ripe fruit; **FAUF:** fatty acids of unripe fruit; **FARF:** fatty acids of ripe fruit; **MEUF:** methyl esters of unripe fruit; **MERF:** methyl esters of ripe fruit; **y:** probit value; ***x:*** log concentration of the sample of the oils, fatty acids, or methyl esters of unripe and ripe fruit.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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