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M Suresh
School of Environmental Toxicology and Biotechnology, Loyola Institute of Frontier Energy (LIFE), Department of Advanced Zoology and Biotechnology, Loyola College, Chennai, Tamil Nadu, India

L Premraj
School of Environmental Toxicology and Biotechnology, Loyola Institute of Frontier Energy (LIFE), Department of Advanced Zoology and Biotechnology, Loyola College, Chennai, Tamil Nadu, India

JMV Kalaiarasi
School of Environmental Toxicology and Biotechnology, Loyola Institute of Frontier Energy (LIFE), Department of Advanced Zoology and Biotechnology, Loyola College, Chennai, Tamil Nadu, India

Correspondence
JMV Kalaiarasi
School of Environmental Toxicology and Biotechnology, Loyola Institute of Frontier Energy (LIFE), Department of Advanced Zoology and Biotechnology, Loyola College, Chennai, Tamil Nadu, India

Bioefficacy and larvicidal activity of *Couroupita guianensis* (Aubl) against housefly, *Musca domestica* (L)

M Suresh, L Premraj and JMV Kalaiarasi

Abstract
The present study was conducted to test the bioefficacy and larvicidal activity of *Couroupita guianensis* (Aubl) against housefly, *Musca domestica* (L). The concentration range used was 62.5, 125, 250 and 500 ppm. Leaves of *C. guianensis* were collected from Loyola College Campus, Chennai, Tamil Nadu, and India during August to December 2016. The plant leaves were dried and coarsely powdered. The plant materials were sequentially extracted with increasing polarity of hexane, chloroform ethyl acetate, and methanol. The dried leaves were pounded mechanically using commercial electrical stainless steel whizzer and extracted using hexane, petroleum ether, chloroform and ethyl acetate, in a soxhlet apparatus (boiling point range: 60°–80 °C) for 8 h separately until exhaustion. All the extracts were concentrated using rotary vacuum evaporator. The residues were weighed and preserved in airtight bottles at 4 °C until further use. The larvicidal activity of the different solvent extracts such as hexane, chloroform, ethyl acetate & methanol of *Couroupita guianensis* were evaluated. The result of this work revealed that the larvicidal effects of the leaf extract of *Couroupita guianensis* on the early third instar larvae of *Musca domestica* were dependent on the concentration of the extracts at 62.5 to 500ppm. Among the extracts ethyl acetate extract of *C. guianensis* presented promisable larvicidal activity with LC_{50} and LC_{90} values of 479.137 and 1969.851ppm against the larvae of *Musca domestica*, respectively. Naturally occurring medicinal plant extracts of *Couroupita guianensis* and leaf-derived compounds merit further study as potential housefly larval control agents or lead compounds.

Keywords: *Couroupita guianensis,* biopesticides, larvicidal, housefly, diseases

1. Introduction

The housefly, *Musca domestica* (L.), is a major inland, curative, veterinary annoyance, machine-driven carriers of bacteriological, helminthic, and virus-related infections [1-3]. More than 100 pathogens are associated with the house flies such as bacteria, protozoans, viruses, and metazoan parasites [4-6] *M. domestica* is reported to spread of *Vibrio cholerae* in a rural shanty town region of India, whereas cohen et al [7] reported 85% decrease in shigellosis and 42% reduction in the incidence of other diarrheal diseases with suppression of flies' population in military camps of Persian Gulf [8].

Housefly abatement primarily depends on continuous applications of synthetic pesticides such as diazinon, fenitrothion, and pyrethrin [9]. Although in effect, their constant use has disrupted natural competitors and controlled the eruptions of some insect species, it resulted in the development of resistance, had adverse effects on non-target organisms, and caused environmental and human health concerns [3, 10-12]. Botanical insecticides are becoming a more popular alternative to synthetic insecticides [12]. The need for evolving novel strategies for controlling the housefly is well highlighted [3].

Medicinal plants may be an alternative source of housefly control agent, because they have been reported to indicate numerous bioactivities such as insecticidal, antifungal, and nematicidal activities [3, 13-15]. In the present scenario, there is an urgent need for alternate eco-friendly pesticides. Plant products have multiple modes of action against different insect pests. Plants are endowed with a potential to harvest an assortment of secondary metabolites like alkaloids, terpenoids, flavonoids, phenols, glycosides, sitosterols, and tannins [16]. These phytochemicals are known to protect the plants from the attack of insect pests [17]. Much effort has, therefore, been turned towards plant extracts or phytochemicals as potential sources of viable housefly resistor agents or as lead compounds [3, 18, 19]

Couroupita guianensis (Aubl.) belonging to the family Lecythidaceae (about 25 genera with 400 species) is a threatened tree worldwide [20, 21, 22]. It is a huge, deciduous, magnificent evergreen tree, growing to a height of 30-35m, with leaves in whorls on the ends of the shoots [23]. This bears a large cluster of flowers in cauliflorous inflorescence with stunning fragrance [24]. The tree is commonly known as Cannonball tree due to its cannon ball like fruits. Naglingam, Ayahuma, Kailaspati, Calabasse Colinetc. are other known names of this plant in India [22, 25]. Parts of the cannon ball tree are widely used in traditional medicine for the treatment of a broad spectrum of diseases. The leaves grow 6" long, are simple with serrate brim; it flowers in racemes; they are coloured yellow, reddish and pink and are stunningly aromatic. Flowers are large 3" to 5", waxy, aromatic, growing directly on the bark of the stem (cauliflory). Fruits are large, globose, arboreal and look like huge eroded cannonballs hanging in clusters, like balls on a string [26].

The *C. guianensis,* whole plant contains phenols as the major component along with alkaloids, flavonoids, stigmasterol, eugenol, linalool, isatin, indirubin, fernesol, nerol, quercertin, saponins, tryptanthrine, indigo, linoleic acid, α- andβ-amirins, carotenoids, sterols, phenolics etc [22, 27-30]. It possesses stupendous biological activities such as antimicrobial, analgesic, antiarthritic, antibiofilm, antidiarrheal, antifertility, antipyretic, antiseptic, antistress antitumor, antiulcer, wound healing, antinociceptive antifeedant, larvicidal, ovicidal and immunomodulatory activities [16, 31]. Methanol extract derived fractions of *C. guianensis* inhibited the development of microorganisms [32]. Petroleum ether and chloroform extracts of this plant exhibited larvicidal activity against vectors [16, 30]. *Couroupita guianensis* leaf extracts revealed antifeedant, larvicidal, and ovicidal activities against *Helicoverpa armigera* and antifeedant activity against *Spodoptera litura* [16, 33-35]. The current study attempted to evaluate the bioefficacy of *Couroupita guianensis* extracts against *Musca domestica.*

2. Materials and methods

2.1 Plant collection and plant extracts

Leaves of *C. guianensis* were collected from Loyola College Campus, Chennai, Tamil Nadu, India during August to December 2016. The plant leaves were dried for 7-10 days in shade at ambient temperature (27-37 oC day time) and coarsely powdered. The plant materials were sequentially extracted with increasing polarity of hexane, chloroform ethyl acetate, and methanol [35].The dried leaves were pounded mechanically using commercial electrical stainless steel whizzer and extracted using hexane, petroleum ether, chloroform and ethyl acetate, in a soxhlet apparatus (boiling point range: 60°–80 °C) for 8 h separately until exhaustion. All the extracts were concentrated using rotary vacuum evaporator. The residues were weighed and preserved in airtight bottles at 4 °C until further use [36].

2.2 Insect rearing

The colonies of *M. domestica* were collected from the poultry farm of the Chennai, Tamil Nadu using a sweep net. The field-collected flies were reared in cylindrical boxes containing a diet of groundnut oil cake and wheat bran (1:3), according to the method described by Kumar et al [37]. Hatched larvae were transferred individually to cylindrical vials (28×12 mm) containing larval diet (constituents: 2 g groundnut oil cake, 5 g wheat bran, 2 g milk powder, 1 g honey mixed with 10 ml of water) which was changed daily until larvae reached the pupal stage. Larvae and pupae obtained through the above-mentioned process were used for bioassays [8].

2.3 Larvicidal bioassay

The larvicidal activity was assessed using the procedure of WHO [38] with some modification and as per the method of Rahuman et al. [39] and Morey et al [40] for the bioassay test. Dipping method was used for this bioassay. Twenty early prepupal larvae were used for this experiment. The desired concentrations of each crude extracts of the plant were prepared in a separate bowl, while one bowl with only the carrier solvent acetone and polysorbate 80 served as control. The larvae were dipped carefully in the solution for 60 s, whereas those for the control were dipped in the bowl containing the carrier solvent and transferred back to the rearing medium. Mortality of these larvae followed by the exposure was monitored after 24 h by touching each larva by a soft, number zero paint brush, and those not responding to the touch were considered dead. For each experiment, five replicates were taken, and each experiment was repeated three times. The concentration range used was 62.5, 125, 250 and 500 ppm. Lethal concentration LC_{50} and LC_{90} was calculated using log probit analysis [41]. Data obtained were subjected to statistical analysis [39, 40].

2.4 Statistical analysis

The larvicidal activity were subjected to probit analysis [41] for calculating LC_{50}, LC_{90} (Table I) and other statistics at 95% fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL); and chi-square values were calculated using software developed by Reddy *et al.* [42]

3. Results

Results of the bioassay of extracts of *Couroupita guianensis* on larval *Musca domestica* is given in Table 1. It can be seen that all extracts were able to cause mortality to larval *M.domestica*. The result of this work revealed that the larvicidal effects of the leaf extract of *Couroupita guianensis* on the early third instar larvae of *Musca domestica* were dependent on the concentration of the extracts at 62.5 to 500ppm. Among the extracts ethyl acetate extract of *C. guianensis* presented promisable larvicidal activity with LC_{50} and LC_{90} values of 479. 137 and 1969.851ppm against the larvae of *Musca domestica*, respectively. Chi-square value was significant at $P<0.05$ level. The result obtained from this study was remarkably significant. The larvae remained alive developed into premature pupae and then adults in the normal control. The larvae of *Musca domestica* exposed to plant extracts showed significant behavioral changes. The most obvious signs of behavioral changes observed in *Musca domestica* was restlessness, loss of equilibrium which finally led to death.

Table 1: Lethal concentration (in ppm) of *Couroupita guianensis* extracts (Ethyl acetate) against the larvae of *Musca domestica.*

Larvae	Extracts	LC_{50} (ppm)	95% Confidence limit LL	UL	LC_{90} (ppm)	95% Confidence limit LL	UL	intercept±SE	slope±SE	χ2
Musca domestica	Hexane	1715.93	1130.16	4483.351	7493.39	3272.793	60371.8	1.4±1.2	2.0±0.4	0.9*
	Chloroform	1035.34	732.488	1992.681	6384.82	2898.918	36995.5	0.1±0.8	1.6±0.3	0.2*
	Ethyl acetate	479.137	388.016	614.503	1969.85	1306.910	4009.153	0.5±0.8	2.0±0.3	4.7*
	Methanol	1167.48	794.195	2559.326	7895.86	3275.216	62409.3	0.2±0.8	1.5±0.3	0.4*

LC_{50} lethal concentration that kills 50% of the exposed larvae, LC_{90} lethal concentration that kills 90% of the exposed larvae, LL lower limit (95% confidence limit), and UL upper limit (95% confidence limit).
$*P \leq 0.05$, level of significance of chi-square values.

4. Discussion

Earlier authors reported that the bio insecticides, particularly those are derived from plant origin, have been increasingly evaluated in controlling the population of insects pest [18, 40, 43-47]. Natural products of plants origin are alternative agents for insect control because they constitute a rich source of bioactive chemicals. Numerous studies have been drawn by considering the toxic effects of plant extracts and dipterans [40, 48-51]. The botanical extracts from the plant leaves, roots, seeds, flowers, and bark in their crude form have been used as conformist pesticides for centuries [36, 37]. *Musca domestica* has been reported to be prone to infection by numerous fungi [56, 57]. Approximately of these naturally infecting pathogens like *B.bassiana* and *M. anisopliae* are midst the utmost infectious pathogens to adult houseflies [58].

In the present study ethyl acetate extracts of *Couroupita guianensis* showed good larvicidal activity (82%) against *Musca domestica* at 500ppm, respectively. These results were corroborate with the findings of Begum et al. [52] who reported good larvicidal activity of crude ethanolic extracts of *Calotropis procera* and *Annona squamosa* against *M. domestica* with LC_{50} values of 282.5 and 550 ppm, respectively. Dried *Libocedrus bidwillii* leaf methonol extract gave 98% mortality of housefly larvae at 100 ppm concentration [53]. Mullai and Jebanesan [59] have reported that the ethyl acetate, petroleum ether and methanol leaf extracts of *C. colocynthis* and *Cucurbita maxima* showed that the LC_{50} values were 47.58, 66.92 and 118.74 ppm and 75.91, 117.73 and 171.64 ppm, respectively, against *C. quinquefasciatus* larvae. Larvicidal efficacies of methanol extracts of *Momordica charantia, Trichosanthes anguina, Luffa acutangula, Benincasa cerifera* and *Citrullus vulgaris* tested with LC_{50} values were 465.85, 567.81, 839.81, 1189.30 and 1636.04 ppm, respectively, against the late third larval age group of *C. quinquefasciatus* [60]. Rahuman et al. [61] have reported that the LC_{50} value of petroleum ether extracts of *J. curcas, Pedilanthus tithymaloides, Phyllanthus amarus, Euphorbia hirta* and *Euphorbia tirucalli* were 11.34, 76.61, 113.40, 424.94 and 5.52 ppm, respectively, against the fourth instar larvae of *C. quinquefasciatus.* The same solvent extract of *R. nasutus* possessed larvicidal effects with LC_{50} values between 3.9 and 11.5 mg/L and *Derris elliptica* showed LC_{50} values of between 11.2 and 18.84 mg/L against *A. aegypti, C. quinquefasciatus, A. dirus* and *M. uniformis* [62]. Seo and Park [3] studied the larvicidal activity of medicinal plant extract from 27 plant species against *M. domestica,* and their result demonstrates that *Phryma leptostachya* shows 100% larvicidal activity against *M. domestica* at 10 mg/g concentration, whereas larvicidal activities of *Atractylodes japonica, Saussurea lappa, Asiasarum sieboldi,* and *Gleditsia japonica* were 89.3, 85.3, 93.3, and 96.6%, respectively at 10 mg/g concentration. Pavela et al. [54] who reported 50% mortality of fourth instar larvae of *M. domestica* at concentrations of 20–50μg for the extracts of *Satureja hortensis*, *Thymus vulgaris*, *Salvia officinalis,* and *M.piperita,* respectively. An extensive assortment of trees and bushes has been found to comprise phytochemical that may be of use in the botanical control of parasites. Miller and Chamberlain [55] reported that an emulsified concentrate of ethanolic ground seed extract of *Azadirachta indica, A. Juss*. blended into cow manure exhibited LC_{50} of 10.5 ppm and LC_{90} of 20.2 ppm for larval houseflies. These results are related to our study.

The finding of this investigation revealed that the leaf extract of *C. guianensis* possesses remarkable larvicidal activity against vector of *M. domestica.* In conclusion, our results presented that ethyl acetate extract from the plant *C. guianensis* showed good activity against *Musca domestica* larval stages. It may be a good alternative source for the synthetic chemicals to control the insect pest.

5. Conclusion

From the present study we conclude that *Couroupita guianensis* (Aubl) proved a promising larvicidal agent against housefly, *Musca domestica* (L) larvae in the laboratory and also reduced egg productions and fecundity.

This revealed the need for development of new insecticides from naturally occurring medicinal plant extracts of *Couroupita guianensis* and leaf-derived compounds merit further study as potential housefly larval control agents or lead compounds.

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