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ORIGINAL RESEARCH

Culex quinquefasciatus Egg Membrane Alteration and Ovicidal Activity of *Cipadessa baccifera* (Roth) Plant Extracts Compared to Synthetic Insect Growth Regulators

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Background: Insecticide resistance among mosquito vectors for synthetic insecticides still remains a major problem for control efforts. This study assessed the ovicidal potential of crude solvent extracts from the medicinal plant *Cipadessa baccifera* comparatively to standard registered synthetic insect growth regulators (IGR) on freshly laid eggs of *Culex quinquefasciatus*.

Method: Five plant extracts were prepared using different solvents. The batches of eggs were exposed to different concentrations of each solvent extract comparatively to synthetic IGR. The hatched eggs of *Cx. quinquefasciatus* were subjected to different concentrations. The first instars that emerged from the eggs were counted daily. The egg hatching inhibition was observed 24, 48 and 72 hrs post treatment. The desiccation median time (DT_{50} and DT_{90}) was calculated.

Results: The percent egg hatching inhibition was inversely proportional to the concentration of extracts. The morphological damage to the eggs was observed. Among five solvent extracts, acetone extracts showed the highest ovicidal activity. The changes in eggshell morphology were observed. The maximum ovicidal activity was observed in acetone extracts with DT_{50} value of 1.70 hrs (0.91–2.22). The methanol plant extract using gas chromatography-mass spectrometry identified 14 compounds.

Conclusion: These results suggest that the acetone extracts of *C. baccifera* have the potential to be used as an ovicidal agent for controlling mosquito populations in aquatic stages. The biodegradability of the extracts has the advantage of being eco-friendly.

Keywords: mosquito, egg hatching, insect growth regulators, ovicidal activity plant extracts

Introduction

Designing innovative vector control tools is of paramount importance due to the development of insecticide resistance among disease vectors.[1] Chemical control is an effective strategy used extensively in vector control for decades. However, the evolution of insecticide resistance among mosquitoes to insecticides has increased in the last two decades.[2]

Culex quinquefasciatus (Say) serves as a vector for filariasis and arboviruses.[3] Human filariasis is a major public health problem and remains a challenging problem socioeconomically in most tropical countries.[4] Insect growth regulators have shown significant larvicidal efficacy against *Aedes albopictus* mosquito at low lethal doses as compared to microbial, organophosphates and synthetic pyrethroid insecticides.[5] Some studies have disrupted hormonal balance inside the developing

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embryo.[6] Su and Mulla reported the ovicidal activity of the neem products such as azadirachtin against *Cx. quinquefasciatus.*[7]

Insect growth regulators are comparatively safer to non-target organisms and have been recommended for mosquito control.[8,9] Insect growth regulators (IGR) include chemicals with a unique mode of actions such as juvenile hormone analog, chitin synthesis inhibitor, ecdysone agonist.[10–12] These IGRs have extended effects to the morphology and physiology of mosquito eggs.[13,14] The surface morphology, physical structure and chemical composition of the eggs determine the ability of eggs to adapt and tolerate adverse conditions such as desiccation.[15] Biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution and low mammalian toxicity.[16] Many herbal products have been used as natural insecticides before the discovery of synthetic organic insecticides.[17]

Cipadessa baccifera Miq. (Meliaceae) is a bushy shrub, distributed in Northern Circars. The active constituents isolated from the seeds of *C. baccifera* include cipadesin, 17a, 20R-dihydroxypregnan-3, 16-dione, 1, 4-epoxy-16- hydroxyheneicos-1, -3, -12, -14, -18.[18]

Therefore, the aim of this study was to evaluate the ovicidal effect and morphological changes in the eggs of *Cipadessa baccifera* extracts using acetone, ethyl acetate, methanol, chloroform and petroleum benzene solvent which was compared with two insect growth regulators (IGRs), against freshly laid eggs of *Culex quinquefasciatus.*

Materials and Methods

Mosquito Rearing

Culex quinquefasciatus mosquito eggs were collected from the National Centre for Disease Control (NCDC), Mettupalayam, Tamil Nadu, India. The eggs were kept in plastic trays containing dechlorinated tap water and maintained at 27±2°C and 75–85% relative humidity under 14:10 light and dark photoperiod. The hatched larvae were reared in dechlorinated tap water in plastic trays and provided with dog biscuits and yeast powder in the ratio of 3:1 as a larval food. Once emerged, the adults were transferred to mosquito rearing cages, holding 10% sugar solution, a food source for adults.

Insect Growth Regulators

The ovicidal efficacy of the two insect growth regulators, a chitin synthesis inhibitor (buprofezin: Buprolord, 25% SC, United Phosphorus Limited, Gujarat) and an ecdysone agonist (Azadirachtin: NeemAzal®, 1% EC, EID Parry India Ltd, India) was determined. The fresh 1% stock solution of each class of insect growth regulator was prepared in dechlorinated tap water. The eggs were exposed to different concentrations (0.1, 0.3, 0.5, 1 and 2 mg/mL).

Plant Materials

The leaves of *Cipadessa baccifera* were collected from Kolli Hills of the Eastern Ghats in the Namakkal district of the Southeast Tamil Nadu (10°12′–11°7′ N, 76° - 77°56 ′E and Altitude 1300 m above sea level). The voucher specimen was numbered and kept in a herbarium for reference.

Preparation of Plant Extracts

The leaves were dried under shade for 7–10 days. The dried leaves were powdered using commercial electrical stainless-steel blender. Three hundred grams of powdered leaves were extracted in five different solvents: chloroform (400mL), ethyl acetate (400mL), acetone (400mL) petroleum benzene and methanol (300mL) in a Soxhlet apparatus (boiling point range 50–80ºC) for 8 hrs. The extracts were concentrated under reduced pressure of 22–26mm Hg at 45ºC and the residues obtained were stored at room temperature.

Egg Exposure to Plant Extracts and Insect Growth Regulators

Freshly laid eggs of *Cx. quinquefasciatus* were incubated at 24 ± 1°C and 75–80% RH for 48 hrs to obtain embryonated eggs, which were treated with different concentrations (0.1, 0.3, 0.5, 1 and 2 mg/mL) of insect growth regulators. The embryonated eggs from the same batch unexposed to any chemicals served as control. The ovicidal effects of insect growth regulators were observed on freshly laid and embryonated eggs of *Cx. quinquefasciatus* (150–200 eggs/raft/replicate). Control experiments were conducted in dechlorinated tap water in three replicates. For ovicidal activity, a slightly modified method of Su and Mulla was performed.[19] The leaf extracts were diluted in the ethanol to achieve various concentrations ranging from 0.1, 0.3, 0.5, 1 and 2 mg/mL. The hatching rates were calculated 48 hrs post treatment by following the formula:

$$\%\ \text{hatchability} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} \text{ X } 100$$

Egg Hatching Inhibition

The first instars that emerged from the embryonated eggs were counted daily. The unhatched freshly laid eggs were observed under a fluorescence microscope for any morphological changes and abnormalities that may have been caused from the exposure to insect growth regulators and plant extracts. The percentage of unhatched eggs in control experiments was adjusted with treatments by using Abbott's formula.[20] The chi-square test was used to determine the significant differences between the proportion of hatched and unhatched egg groups ($p< 0.05$).

GC-MS Analysis and Identification of Compounds

The plant extract of *C. baccifera* was analysed by gas–liquid chromatography (Polaris Q Ion Trap GC/FID) and mass spectrometry (PerkinElmer Q-700 equipment). The methodology developed by Cheng et al was used.[21]

Statistical Analysis

Data were subjected to Probit analysis in SPSS version 25 (IBM Corp., Armonk, NY, USA). Desiccation median time DT_{50} (defined as the duration at which 50% of eggs were desiccated) and median desiccation time DT_{90} (defined as the duration at which 90% of eggs were desiccated) of the eggs were calculated. Comparisons between proportions of DT_{50} and DT_{90} were computed using the chi-square test.

Results

Effects on Embryonic Development

The acetone extract of *C. baccifera* produces morphological changes in the eggshell, resulting in damage to the egg membrane and decreased egg hatchability (Figure 1). No significant relationship between the rates of egg hatching inhibition and abnormalities of egg morphology was observed in treatments using IGR.

Egg Hatchability in Insect Growth Regulators

The hatchability of freshly laid eggs of *Cx. quinquefasciatus* in insect growth regulators was also found to be dosage dependent. The egg hatching inhibition of *Cx. quinquefasciatus* was higher in eggs exposed to

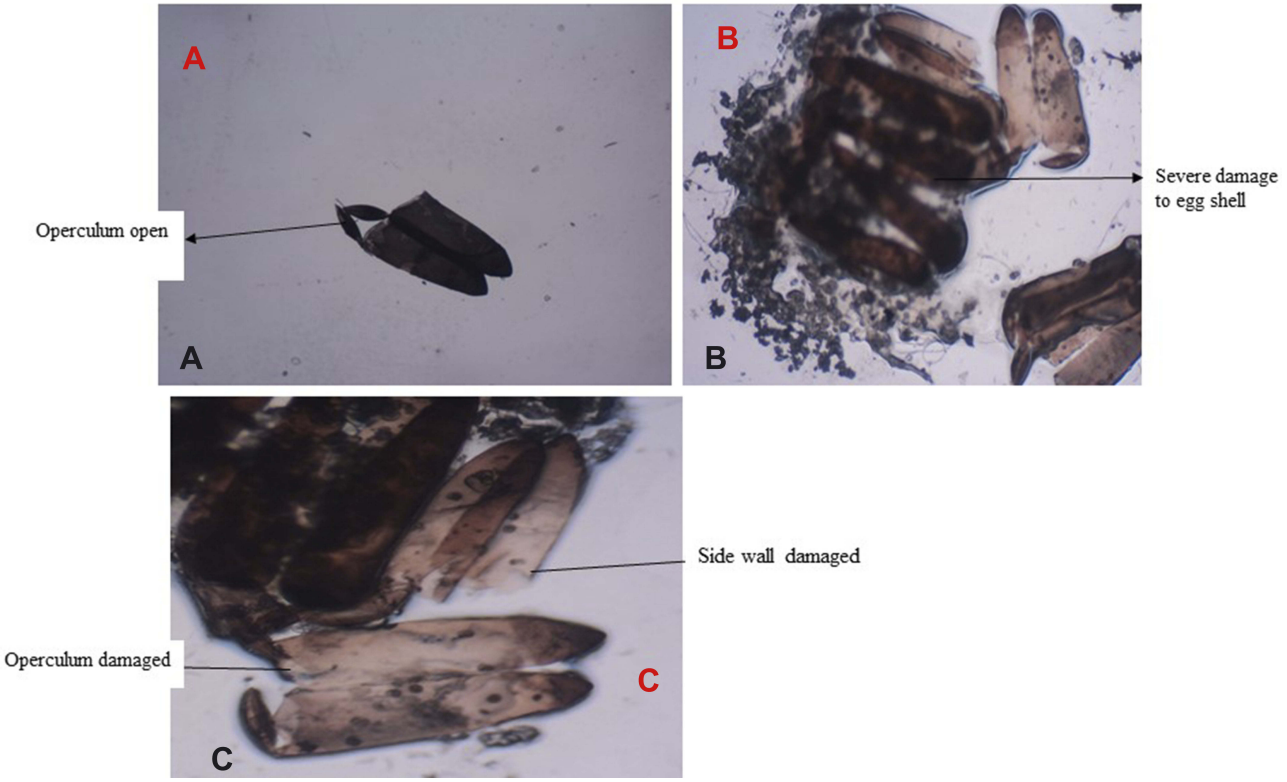

Figure 1 (**A**)Control Operculum open and egg hatching. (**B** and **C**) Abnormal egg hatching and operculum open from side wall damaged after exposure to *Cipadessa baccifera* acetone plant extract.

azadirachtin (75%) than in buprofezin (55%) at 0.1mg/mL concentration, and the trend was also observed in other higher concentrations.

Egg Hatchability in Plant Extracts

Among the plant extracts tested for egg hatchability inhibition, the acetone extract of *C. baccifera* exerted 98% egg hatching inhibition at a concentration of 0.1 mg/mL, whereas higher concentration resulted in 100% eggs hatching inhibition. In the control arm, egg hatchability was 100%.

Effects on Egg Hatching Patterns

Egg hatching pattern in azadirachtin and buprofezin was similar to that of control, while that of freshly laid eggs of *C. baccifera* extracts was dosage dependent. The median desiccation time (DT_{50}) of *Cx. quinquefasciatus* eggs was observed to be higher for acetone extract (1.70 hrs). There was no significant difference in hatching inhibition of freshly laid eggs at any concentrations when exposed to other solvent extracts of *C. baccifera* ($P > 0.05$) (Table 1).

GC-MS Analysis

The methanol plant extract using gas chromatography-mass spectrometry identified 14 compounds in the *C. baccifera* plant chemical constitutions. The major peaks were 4H-1-benzopyran-4-one,3,5,7-trimethoxy-2-(3,4,5-trimethoxyphenyl) (41.6%), palmitic acid vinyl ester (5.7%), octadecanoic acid,1-[[(1-oxohexadecyl) oxy]methyl]-1,2-ethanediyl ester (8.4%), octadecanoic acid,1-[[(1-oxohexadecyl)oxy]methyl]-1,2-ethanediyl ester (5.6%), cyclopropane carboxylic acid, and 2-methyl-, 2,6-di-*t*-butyl-4-methylphenyl ester (5.4%) (Table 2).

Table 1 Estimation of Desiccation Time of *Culex quinquefasciatus* Eggs

Plant Extracts and IGRs	DT_{50} hrs (LCL-UCL)	$DT_{90\ hrs}$ (LCL-UCL)	$\chi 2$	df
Acetone	1.70 (0.91–2.22)	2.82 (2.32–3.25)	1.085	3
Ethyl acetate	4.40 (3.90–5.00)	6.32 (5.90–7.10)	0.321	3
Methanol	5.10 (4.90–5.55)	6.34 (5.34–7.00)	2.120	3
Chloroform	3.80 (3.24–4.11)	5.43 (4.32–6.10)	5.870	3
Petroleum benzene	7.56 (6.30–8.10)	9.32 (8–10.12)	2.832	3
Azadirachtin	2.11 (1.09–2.81)	3.90 (2.87–4.11)	0.431	3
Buprofezin	4.92 (3.73–6.01)	7.33 (5.98–8.55)	0.821	3

Notes: Significant differences among acetone plant extracts for individual concentrations at $^*P < 0.05$; Lower–upper fiducial limits were shown in parenthesis for respective DT_{50} and DT_{90}.

Abbreviations: DT_{50}, median desiccation time; DT_{90}, desiccation time for 90% eggs, $\chi 2$, Chi-square.

Discussion

The findings of the current study have shown that plant extracts have the potential to contribute in alternative pesticides for disease vector control. The environment is an incomparable reservoir of natural products that exhibit structural features, which have not been found in terrestrial natural products.[22] Several studies have demonstrated that plants are an excellent source of components with biological activity such as antifungal, antiviral, phytotoxic and larvicidal activities.[23–28] The screening of medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product-based mosquito abatement practices.[24–32]

Plants may serve as a suitable alternative source of insecticides to complement the toolbox of available synthetic insecticides for the future. Due to their low mammalian toxicity, the crude extracts have many active compounds with different acting mechanisms, most inexpensive and are readily available in many areas of the world.[33] Different parts of plants contain complex chemical composition with unique biological activity which is thought to be due to toxins from different secondary metabolites, which may act as mosquitocidal agents.[33] The plant crude extracts seemed to be more effective than single isolated compound which might be attributed to the natural synergistic effect that acts in many different ways to inhibit the development of an embryo in eggs. The results of this study showed that the acetone extract of *C. baccifera* showed 98% egg hatching inhibition (2% hatchability) was recorded at 24, 48 and 72 hrs at 0.1, 0.3, 0.5, 1 and 2 mg/mL, respectively. It was also found that the acetone extracts of *C. baccifera* damage the eggshell and subsequently increases hatching inhibition.

Chitinous egg development was affected in buprofezin-treated embryos, and probably, pharate larvae used the body pressure for egg hatching. Our results showed less toxicity to egg hatching and embryo development when exposed to buprofezin and azadirachtin. In contrast, buprofezin and azadirachtin substantiate their role in altering hormonal actions during embryonic development of the *Culex* eggs rather than altering egg hatching process.[34,35] Suman et al have suggested that less toxicity to larvicidal and ovicidal efficacies are governed by the type and concentration of different classes of insect growth regulators.[14]

Table 2 Chemical Composition of Methanol Leaf Extract from *C. baccifera*

S/No	RT	Area	Area %	Compound Name	Activity
1	12.883	9,756,920.0	1.203	Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-	Insecticidal activity
2	19.440	7,510,873.0	0.926	Phytol	Insecticidal and antibacterial property
3	22.646	9,179,309.0	1.132	5-(2,5-Dimethoxy-phenyl)-2H-pyazol-3-ol-	Antioxidant and anti microbial activity
4	23.312	14,056,956.0	1.733	2H-1-benzopyran-2-one,6-(1-hytroxy-3-methylbutyl)-7-methoxy-	Insecticidal activity
5	24.307	44,122,588.0	5.441	Cyclopropanecarboxylic acid, 2-methyl,-,2,6-di-t-butyl-4-methylphenyl ester	Insecticidal and acaricidal activity
6	27.683	38,265,564.0	4.719	3-Methyl-2-(2-oxopropyl) furan	Anticancer activity
7	28.114	33,638,632.0	4.148	Octadecanoic acid, ethenyl ester	Insecticidal activity
8	29.174	45,929,464.0	5.664	Palmitic acid vinyl ester	Antifungal activity
9	29.399	123,963,688.0	15.287	Unknown	Unknown
10	29.589	336,982,432.0	41.556	4H-1-benzopyran-4-one,2-(3,4-dimethoxyphenyl)-3.5,7-tetrmethoxy-	Larvicidal and antibacterial activity
11	29.979	14,258,184.0	1.758	Palmitic acid vinyl ester	Antifungal activity
12	30.079	19,795,344.0	2.441		No known activity
13	30.845	68,102,568.0	8.398	Octadecanoic acid, 1-[[1-oxohexadecyl)oxy]methyl]-1,2-ethanediyl ester	No known activity
14	31.120	45,352,300.0	5.593	Octadecanoic acid, 1-[[1-oxohexadecyl)oxy]methyl]-1,2-ethanediyl ester	No known activity

In the present study, *Cx. quinquefasciatus* eggs were most susceptible to acetone plant extract of *C. baccifera* as compared to the two IGRs. Previous studies reported that diflubenzuron, pyriproxyfen and azadirachtin were less toxic to *Cx. quinquefasciatus* eggs at a WHO-recommended concentrations.[7,8,36] In order to manage mosquito populations at the egg stage, the eggs need to be exposed at higher concentrations of insect growth regulators for shorter rather than long durations. Freshly laid eggs are more vulnerable to the toxicity of insect growth regulators than embryonated eggs. Similar observations using insect growth regulators were made by Miura et al.[36,37] Miura and colleagues suggested hat, freshly laid and 2–14-hr-old eggs were more vulnerable to diflubenzuron exposure.[37] Meanwhile, Vasuki demonstrated species-specific variation on ovicidal action of chitin synthesis inhibitor and juvenile hormone analog against eggs of *An. stephensi* (Liston), *Cx. quinquefasciatus* and *Ae. aegypti*.[36] These differences may be attributed to the inability of insect growth regulators to disrupt hormone actions during egg development and the loss of shell permeability due to endochorion tanning and wax layer formation.[38] The interspecific variations in *Ae. aegypti* eggs from different habitats have shown differences in the desiccation of survival time.[39] Moreover, this study could not find any relationship between eggs and ovicidal activity of insect growth regulators based on egg desiccation time, and this could be one of the reasons for differential ovicidal efficacy of insect growth regulators.

The isolation of compounds from leaf extracts of *C. baccifera* could lead to the development of natural mosquitocidal products to complement the synthetic insecticides. The use of natural plant-based products by individuals and communities would generate local employment, reduce dependence on expensive imported synthetic products and stimulate local efforts to enhance public health.[29] For example, the essential oil extracted from

Citronella and *Eucalyptus* provides the active ingredients of some commercial repellents. Such substances are sold under several brand names.[40]

Limitation of the Study

This study had no resources to study other mosquito families' response to these extracts.

Conclusion

This study has shown that the acetone extract of *Cipadessa baccifer*, possesses ovicidal activity on *Cx. quinquefasciatus* eggs at very low concentrations, but no significant activity, eggshell morphology and ovicidal efficacy were observed in insect growth regulators. These results open the possibility for further investigations of the efficacy of growth inhibition properties of plant-based extracts.

Ethics Approval

This study was approved by the Indian Council of Medical Research and Periyar University ethics committees.

Availability of Data and Materials

The data related to the conclusion of this study have been included in this manuscript.

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Disclosure

The authors report no conflicts of interest in this work.

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