Research Article

Effect of *Solanum lycopersicum* leaf extracts against larvicidal activity of *Aedes aegypti* L.

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ABSTRACT

Introduction: *Aedes* mosquitoes are responsible for transmitting various life-threatening diseases all over the world and created a burden to society. Control of mosquito vectors is the key challenge to avoid disease transmission. In this regard, the present work is focused to utilize the agronomical waste of *Solanum lycopersicum* leaf extracts and to evaluate the larvicidal activity against *Aedes aegypti*.

Methods: The qualitative and quantitative screening of *S. lycopersicum* leaf extracts were carried out. The larvicidal activity of different concentrations were assessed against 3rd instar *Ae. aegypti* and to determine the morphological effects. The chemical constituents were analysed by gas chromatography coupled with mass spectroscopy (GC-MS).

Results: The preliminary phytochemical screening of *S. lycopersicum* leaf extracts revealed the presence of alkaloids, saponins, phenols and flavonoids. The methanol extract exhibited strong larvicidal activity at 48h treatment with an LC_{50} value of 20.323mg/ml. The morphological changes revealed that abnormal movement and coiling of treated larvae at 24h. Furthermore, severe damage was noticed in the digestive and respiratory tract of *Ae. aegypti* larvae at 48h, later on complete mortality was observed. The GC-MS analysis of methanol extract showed chemical constituents such as phytol acetate (42.66%), neophytadiene (29.38%) and other minor compounds.

Conclusion: Based on the results, it can be concluded that methanolic leaf extract of *S. lycopersicum* could be an alternative source to control mosquito vectors and further investigation is strongly suggested in order to utilise this source in many disease-endemic areas.

Keywords: *Solanum lycopersicum; Aedes aegypti;* phytochemicals; GC-MS; larvicidal activity

INTRODUCTION

Aedes mosquitoes are the primary vectors that pose a severe threat to human life and animals. The rapid growth of the mosquito population leads to severity and prevalence of mosquito-borne diseases such as chikungunya, dengue, yellow fever and zika (1). The platform for such diseases could be lack of cleanliness and awareness among the people, globalisation of travel and trade, unplanned urbanisation, drastic environmental changes, deforestation and industrialised farming (2). The impact of these factors on disease transmission in emerging countries may end up in social disruption and economic imbalance (3). Millions of death cases were reported every year only because of mosquito-borne diseases. Recently, Vector Control Disease International agency (VCDI) has reported that mosquito-borne diseases are increasing rapidly (4). The two major vectors, *Aedes aegypti* (*Ae. aegypti*) and *Aedes albopictus* are known to spread dengue, chikungunya and zika. *Aedes* species are highly anthropophilic, frequently bites and thrives close to humans (5).

Currently, there are no proper vaccines to treat mosquito-borne diseases. Mosquitoes have dragged considerable attention of young researchers worldwide (6). Targeting the vector is the only practical approach in order to control and reduce the disease. Various stages (egg, larvae, pupa and adult) can be targeted in the life cycle of mosquito, however larval stage is most preffered in Integrated Mosquito Management (IMM) (7). Continuous use of synthetic insecticides resulted in adverse environmental effects and developed insect resisitance (8). So, this provokes the researchers to develop new, economic and environmentally healthier vector control methods. During the past few decades, many natural products have been examined against mosquito vectors and suggested as a possible alternative source over the synthetic chemical insecticides (9). Most plant products and microbial organisms have been reported as natural biological control agents to minimise mosquito populations (10, 11).

Tomato (*Solanum lycopersicum*) is the most cultivated plant among Solanaceae and widely used in culinary. The Solanaceae, commonly known as nightshade, includes over 2500 species among the plant families and is well documented for its phytochemical compounds. Solanaceae plants are distributed extensively in temperate and tropical regions (12).

Solanum showed several biological activities such as antimycotic, molluscicidal, antiviral and cytotoxic properties (13). They are extensively used for biological and pharmacological activities due to the presence of rich secondary metabolites (14). *Solanum* considered as a model for morphological, physiological and genetic studies because of its genetic and molecular advantages (15). Meanwhile, Solanaceae are traditionally used to treat heat burning, ulcers, rheumatism, eye diseases, heart ache and many pharmacological activities such as antiseptic, anti-inflammatory, antidysenteric, antitumor, anticancer and antimalarial (16). Similarly, the fruit and seed extracts of *S. lycopersicum* evaluated for larvicidal activity (14). However, to the best of our knowledge, there is no data available from the leaves of *S. lycopersicum* for larvicidal activity against *Ae. aegypti*. The leaves of tomato are waste after harvesting the fruit which can be utilised for the development of effective larvicide that may help to overcome synthetic one. Therefore, the current study was designed to assess the larvicidal activity of *S. lycopersicum* leaf extracts against third instar larvae of *Ae. aegypti* under laboratory conditions.

MATERIALS AND METHODS

Plant material and preparation of crude extracts

The fresh leaves of *S. lycopersicum* collected from an agricultural field (latitude 14.259775°N, longitude 76.864070°E) in Chitradurga district during October 2019, Karnataka, India and the voucher specimen is stored in the Department of Applied Botany, Mangalore University, Mangalagangothri. The leaves were washed under running tap water followed by distilled water to remove all the dirt and debries. Shade dried and grounded into coarse powder using mechanical blender. The plant powder (50 g) was sequentially extracted using petroleum ether and methanol by soxhlet apparatus. Additionally, the aqueous extract was prepared by soaking leaf powder (20g) in distilled water (100 ml) for about 24h at room temperature and filtered using whatmann no. 1 filter paper. The filtrates were kept in the water bath for evaporation until it becomes dark greenish mass and stored below 5°c for further experiments. Finally, the percent of the yield of each extract was calculated.

Qualitative and Quantitative screening

The qualitative phytochemicals of each crude extracts were screened according to standard protocol (17). The quantitative estimation of total alkaloids (18), saponins(18), phenolics (19) and flavonoids (20) was carried out with slight modifications.

Mosquito colony rearing

The mosquito colony (*Ae. aegypti*) rearing was carried out in National Institute of Malaria Research Centre (NIMR) Field Unit, Bangalore, Karnataka, India. The *Ae. aegypti* larvae were placed in a white tray containing Reverse osmosis (RO) water and kept at 27 ± 2°C with RH 75% to 85% and 14L:10D photoperiod cycles. Larvae fed with powdered Brewer's yeast and dog biscuit in the ratio 3:1. Pupae developed within 6- 8 days were transferred to cages for adult emergence. The emerged adult colony is provided with cotton balls dipped in 10% glucose and soon after three days of emergence, mosquitoes were allowed to blood feed using Wistar albino rats. The ovi-traps containing water was placed in the cage after two days of a blood meal (21).

Bioassay of larvicidal activity

The larvicidal activity of petroleum ether, methanol and aqueous extract of *S. lycopersicum* leaves were evaluated as per the World Health Organization (WHO) guidelines with slight modifications (21). Solvent extracts were dissolved in ethanol and aqueous extract was dissolved in deionised water for experimental studies. Twenty five 3rd instar larvae were introduced into individual cup containing 199 ml of distilled water and 1 ml of the desired solution of plant extract (10-50mg/ml concentrations). The test was conducted in triplicates and larvaes were not provided with food during the test. The larval mortality was observed and recorded after 24h and 48h of post-treatment, where ethanol was served as control. The percentage mortality is recorded as mean values of triplicates. The dead and moribund larvae were counted. Mortality rate was corrected using Abbott's formula (21).

Microscopic analysis

After 24h treatment, treated larvae were randomly collected from the test cup, placed on a sterile glass slide using a thin brush to examine morphological changes. The larval slides were observed under light microscope at room temperature (22).

GC-MS Analysis

The crude methanolic leaf extract was analysed via Shimadzu gas chromatograph-mass spectrometer (Model Number: QP2010S) equipped with a Rxi-5sil MS column (30m x 0.25mm x 0.25m film thickness). Temperature program set from 80°C (1 min) to 280°C (20 min) and helium was used as carrier gas. Injection volume was 1.0 µl and the inlet pressure of 65.2 kPa was maintained. Linear velocity (u) maintained up to 36.8 cm/sec. Based on the retention indices, compound identification was made using a GC-MS. Percentage composition of the compounds was determined by The NIST 11 and WILEY 8 libraries (23).

Statistical Analysis

The experimental data expressed as Mean ± SEM and larvicidal activity was assessed by the Log- probit regression model for lethal concentration value using MS office version 2019. The recorded mortality data was subjected to analysis of variance (ANOVA) and

data expressed as a mean of triplicates in each concentrations. One way ANOVA followed by Dunnett T3 test was used to analyse the significant effect of extracts on *Ae. aegypti* using SPSS software, version 20; values were considered as statistically significant when $p < 0.05$.

RESULTS

Qualitative and Quantitative analysis

The percent yield of *S. lycopersicum* plant extracts of petroleum ether, methanol and aqueous was 7.78, 26 and 13% respectively. The preliminary phytochemical investigation revealed the presence of alkaloids, phenolics, saponins and flavonoids in extracts of *S. lycopersicum* (Table 1).

Table 1: Phytochemical screening of *S. lycopersicum* leaf extracts

Tests for	Petroleum ether	Methanol	Aqueous
Alkaloids	+	+	+
Terpenoids	–	+	–
Glycosides	–	–	–
Phenolics	+	+	+
Tannins	–	+	+
Saponins	–	+	+
Flavanoids	–	+	+
+ = Present, - = Absent			

The quantitative estimation of *S. lycopersicum* leaf extracts resulted in 8.8% of total alkaloid content and 44.6% of saponins in dry plant powder. The phenolic contents were determined using gallic as standard and expressed as mg of Gallic Acid Equivalent/g of tissue. The highest phenolic content was in methanol extract (228.80±0.005 GAE/g) compared to petroleum ether (180.236±0.002 GAE/g) and aqueous extract (182.27±0.006 GAE/g). Furthermore, the flavonoids content were determined using quercetin as standard. Flavanoid contents were 771.40±0.005 and 247.33±0.003mg quercetin equivalent/g of methanol and petroleum ether extracts, respectively.

Larvicidal bioassay

The larvicidal activity of crude petroleum ether, methanol, aqueous extracts in different concentrations of *S. lycopersicum* leaves against third instar larvae against *Ae. aegypti* are represented in Table 2. The median lethal concentration (LC_{50}) value of extracts were 20.323mg/ml, 40.457mg/ml and 45.919mg/ml for methanol, petroleum ether and aqueous, respectively. The plant extracts showed significant ($p < 0.05$) larvicidal toxicity against *Ae. aegypti* larvae. Abnormal movement and coiling of treated larvae was observed in methanol extract of treated cups, where they showed distinct morphological changes after 24h of treatment. After 48 hrs, complete (100%) mortality occurred in methanol extract, whereas in petroleum ether (43%) and aqueous extracts (36%) moderate larvicidal activity was observed. The active extract was further analysed using GC-MS analysis to identify the active compound. Fig. 1 represents the percentage of mortality against *Ae. aegypti* larvae. The mortality was dependent on the concentration of extracts. In control, no mortality was observed. The result indicates the significant variations ($p < 0.05$) in treated and control larvae.

Table 2: Larvicidal activity of *S. lycopersicum* leaves extract against *Aedes aegypti* larvae

Extracts	Concentrations (mg/ml)	Larval mortality	LC_{50} Values (mg/ml)	95% confidence limit		R^2 Value
				UCL	LCL	
Control		1.00 ± 0.57				
Methanol	10	1.00 ± 0.57	20.323	27.952	16.409	0.905
	15	8.00 ± 0.57*				
	20	13.00 ± 0.57*				
	25	17.00 ± 1.52*				
	30	18.00 ± 1.15*				
	35	20.00 ± 1.52*				
	40	23.00 ± 2.00*				
	50	25.00 ± 0.00*				
Petroleum ether	00	0.33 ± 0.33	40.457	48.80	37.876	0.756
	10	0.00 ± 0.00				
	15	1.33 ± 0.88				
	20	4.33 ± 0.88				
	25	5.00 ± 0.57*				
	30	7.00 ± 1.15				
	35	8.00 ± 0.57*				
	40	10.33 ± 0.88*				
	50	10.66 ± 1.20				
	00	0.33 ± 0.33	40.919	54.510	42.329	0.857
	10	0.00 ± 0.00				
	15	0.33 ± 0.33				
	20	1.00 ± 0.57				

Aqueous	25	2.33 ± 0.33				
	30	3.00 ± 1.00				
	35	5.33 ± 0.88				
	40	7.66 ± 0.66*				
	50	8.66 ± 0.88*				

Data provided as mean ± SEM (n = 3) (Anova/Dunnett T3 test); *p< 0.05 indicates significant variations between the control and extracts. UCL: upper confidence limit, LCL: lower confidence limit, R^2: Chi square value.

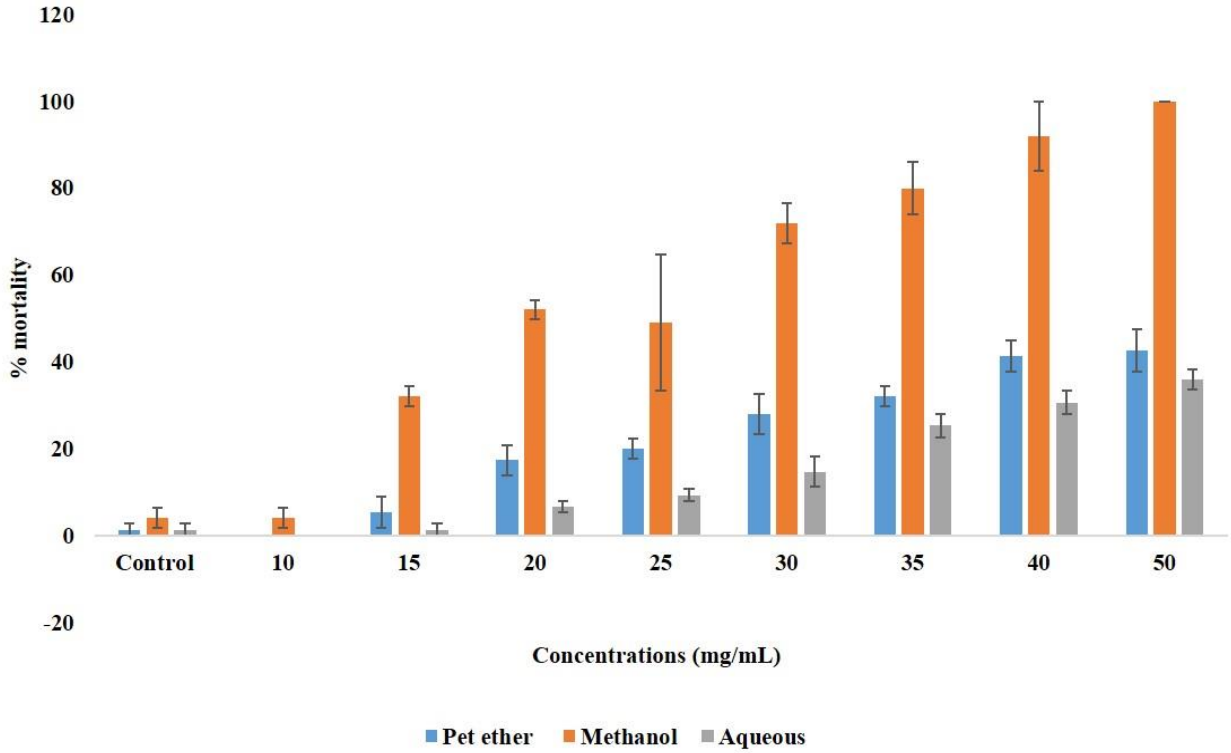

Fig. 1: Mean mortality (%) of larvicidal activity of petroleum ether, methanol and aqueous extracts of *Solanum lycopersicum* leaves against *Aedes aegypti* larvae.

Morphological changes

After 24 h, the entire body of treated and untreated larvae was observed under a light microscope. The larvae treated with methanol extract of *S. lycopersicum* was severely damaged. By visual observation, abnormal body movement and coiling of treated larvae was noticed in methanol extract. They were showing distinct morphological changes resulting in complete damage of digestive (oozing out of digestive tract content) and respiratory tract along with the shrunken head with dark spots when observed under light microscope. No morphological changes were detected in control larvae (Fig. 2 and 3).

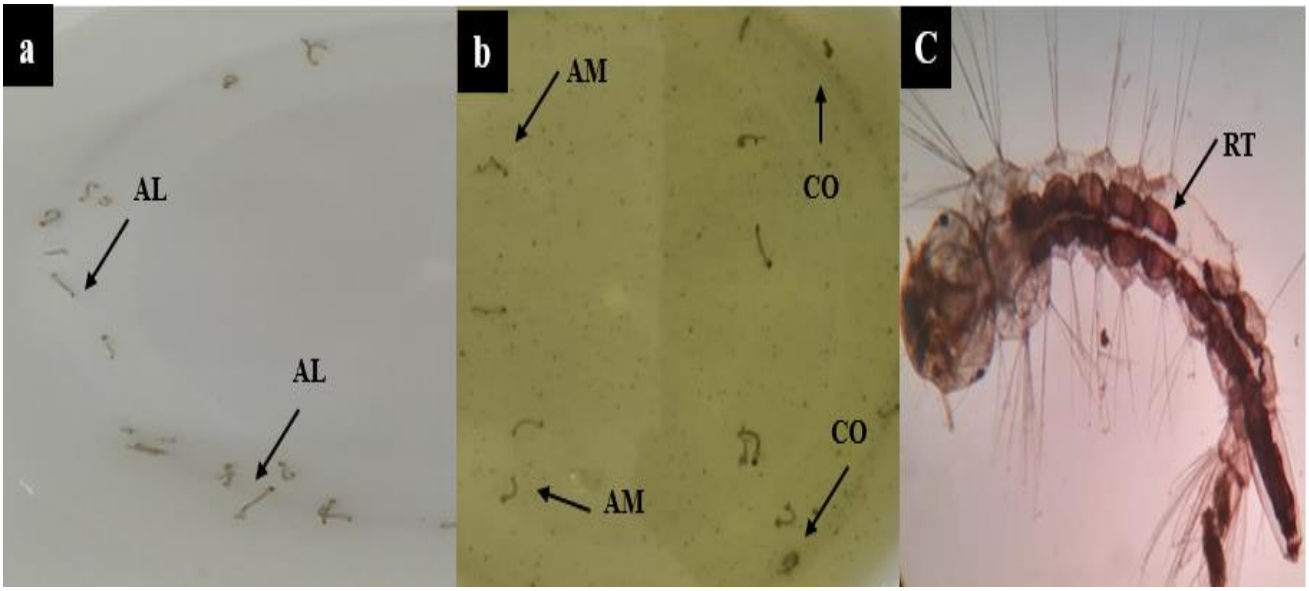

Fig. 2: Visual observation of untreated and *S. lycopersicum* treated larvae. a. Control, b. Test, c. larvae under light microscope. AL- Active larvae, CO- coiling, AM-abnormal movement, DT- damaged digestive tract.

Fig. 3: Microscopic observation of treated and untreated *Aedes aegypti* larvae. A. Control larval - Head (H), B. Complete *Ae. aegypti* larvae, C. Treated larvae showing damaged Head, D. Damaged Respiratory tract (RT), E. Severe damage in the abdominal region, F. Digestive tract (DT), Respiratory tract (RT) and Anal gills(AG) damages.

GC-MS report analysis

The chemical composition of crude methanol leaf extract of *S. lycopersicum* was evaluated by GC-MS. Compound name, molecular weight, molecular formula, retention time, peak area, pharmacological action were summarised in Table 3. The identified constituents were phytol acetate (42.66%), neophytadiene (29.38%), 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (10.01%)) and methyl palmitate (7.39%) as major while linolenic acid (4.78%), cholesterol (2.87%), methyl octadeca-9, 12 dienoate (2.08%) and methyl stearate (0.83%) are present in minor quantity.

Table 3: GC-MS analysis of methanol leaves extract of *Solanum lycopersicum*

Sl no.	Compound name	Molecular formula	Molecular weight (g/mol)	Retention time	Area Peak (%)	Similarity percentage	Group	Pharmacological action
1	Neophytadiene	$C_{20}H_{38}$	278.5	26.366	29.38	95	Diterpenoids	Antipyretic, Analgesic, and Anti-inflammatory, Antimicrobial, Antioxidant (24)
2	Phytol acetate	$C_{22}H_{42}O_2$	338.6	26.868	42.66	91	Diterpenoids	Anti-inflammatory, Antileishmanial and Antitrypanosomal (25)
3	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	$C_{20}H_{40}O$	296.5	27.241	10.01	95	Diterpenoids	-
4	Methylpalmitate	$C_{17}H_{34}O_2$	270.5	28.166	7.39	96	Fatty acid methyl ester	Acaricidal activity (26)
5	Methyl octadeca-9,12-dienoate	$C_{19}H_{34}O_2$	294.5	31.354	2.08	94		-
6	Linolenic acid	$C_{19}H_{32}O_2$	292.4	31.463	4.78	93		-
7	Methyl stearate	$C_{19}H_{38}O_2$	298.5	31.977	0.83	88		-
8	Cholesterol	$C_{27}H_{46}O$	386.654	47.507	2.87	87	Phyto sterol	-

Total Percentage	100		

DISCUSSION

The present study identified *S. lycopersicum* methanol leaf extract as a promising larvicidal agent against third instar larvae *Ae. aegypti*. The petroleum ether and aqueous extracts of *S. lycopersicum* leaves did not exert any good results. However, methanol extract showed an appreciable larvicidal effect on the growth of *Ae. aegypti*. The present study has conformity with other reports available in the literature with varied results among different mosquito species. Molan *et al.,* reported that seed and fruit extract of *S. lycopersicum* was effective against *Culex quinquefasciatus* at 100mg/ml (14). Furthermore, the hydroethanolic extract of fresh and old leaves of *S. lycopersicum* showed potential results against *Aedes* species at 200-250 ppm (27). The activity of the natural extract against the larvae can vary depending on the plant parts, age of species (young, senescent), solvent used [9], season (28) and geographical distribution (29). The Chloroform : methanol extract (1:1 v/v) of *Solanum villosum* leaves extract exhibited toxic effect against *Cx. quinquefasciatus* (30). Likewise, hexane and aqueous extract of *Solanum nigrum* were 100% effective at 100 and 1000 ppm for all the tested larva's (*Anopheles culicifacies* species A, *An. culicifacies* species C, *An. stephensi*, *Cx. Quinquefasciatus*) except for *Ae. aegypti* (96%) (31). The methanol extract of orchid *Sarcanthus pauciflorus* showed 100% mortality at 0.5 mg/ml (32). Similarly, the larvicidal activity of leaf extracts of *Adhatoda vasica*, *Centella asiatica*, *Holarrhena antidysentrica*, *Hugonia mystax*, *Mentha spicata* against 3rd instar larvae of *Aedes aegypti* L. and *Cx. quinquefacsiatus* were toxic (33).

The mode of action in the plant secondary metabolites on larvae is not clear. The past investigations demonstrated that the plant-based phytochemicals affect the midgut of larvae and it also damages the enzyme acetylcholinesterase (22, 34). The treated larvae of *Ae. aegypti* with *Arachis hypogea* peel and *Magnolia denudata* seed extracts showed severe damaged columnar epithelial cells, brush border cells, shrunken body segments and intoxication in the gastric caecum. Similarly, the pleiotropic membrane surrounding the lumen was ruptured and oozing of midgut content was observed in treated larvae (22, 35). Likewise, ethyl acetate extract of *Penicillin daleae* showed morphological deformities in the *Ae. aegypti* larvae (36). Most of the chemical compounds present in *S. lycopersicum* were reported earlier and has been screened for many pharmacological assays as mentioned in Table 3 (24-26).

Phytochemicals play a vital role in diseases control and considered as eco-friendly medicine. Secondary metabolites produced against predators are the key ingredient in controlling mosquitoes. The phytochemical constituents like phenolics, alkaloids and terpenoids may jointly or independently contribute in destroying the mosquitoes as either larvicidal or oviposition deterrent or repellent agents (37).

CONCLUSION

To sum up, methanolic leaves extract of *Solanum lycopersicum* exhibited potential larvicidal activity against third instar larvae of *Aedes aegypti*. Plant-based agents give a better option with eco-friendly and low cost approaches over synthetic one. Based on the results, it can be concluded that the toxic content present in *S. lycoperscium* leaves was able to damage the larval organs. The GC-MS analysis strongly provide evidence to support larval mortality by showing terpenoid compounds such as phytol acetate and neophytadiene as major constituents. Furthermore, studies on isolation of active compound, mode of action at a particular site and toxicological aspects are under progress. The author strongly suggests to utilize agro-wastes in order to design and develop eco-friendly larvicidal agents which could be probed for *Aedes aegypti* mosquito control.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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