International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 30-04-2024, Accepted: 20-05-2024, Published: 14-06-2024
Volume 9, Issue 6, 2024, Page No. 53-57

Culminating efficacy: Exploring the smoke toxicity and repellent properties of gloriosa superba leaf extract against anopheles stephensi and culex quinquefasciatus mosquitoes

Karunesh Singh[1*], Indu Singh[2]
[1] Department of Zoology, D.A.V.P.G. College Azamgarh, Uttar Pradesh, India
[2] Department of Zoology, K.N.I.P.S.S. Sultanpur, Uttar Pradesh, India
Corresponding Author: drkaruneshsinghmaniyarpur@gmail.com

Abstract
This study investigates the repellent activities and smoke toxicity effects of the petroleum ether seed extract of Gloriosa superba against Anopheles stephensi and Culex quinqufasciatus *mosquitoes*. Repellency tests were conducted at different concentrations (10ppm, 15ppm, 20ppm, 25ppm, and 30ppm) and time intervals (5.00-6.00 pm to 9.00-10.00 pm). Results demonstrated a concentration-dependent reduction in mosquito bites, with the highest concentration (30ppm) consistently exhibiting the most significant repellent effect. Anopheles stephensi showed a range of protection from 10.26% to 70.84%, while *Genus*-Culex exhibited protection from 16.17% to 95.53%. Smoke toxicity effects were evaluated using petroleum ether seed extract amended *mosquito coils against adult* Anopheles stephensi and Culex *quinquefasciatus.* The coils demonstrated varying degrees of fed, alive, dead and *unfed mosquitoes*, with CD (5%) values indicating significant differences in toxicity levels. Additionally, the study assessed the smoke toxicity effects of Gloriosa superba extracts on the reproduction and survival of both mosquito species, revealing varied impacts among different coils. These findings provide comprehensive insights into the potential of Gloriosa superba as a natural mosquito repellent and highlight its multifaceted effects on *mosquitoes* behavior and survival.

Keywords: Gloriosa superba, mosquito repellent, smoke toxicity, coil assay, survival rate

Introduction
Mosquito-borne diseases for Human population, particularly in all regions (Lim *et al.*, 2023) [6]. Mosquito-borne diseases for mosquito control strategies. Among the myriad approaches, plant-derived compounds have gained prominence for their potential as eco-friendly alternatives to conventional insecticides. In this study, we delve into the multifaceted effects of *Gloriosa superba* petroleum ether seed extract, specifically examining the smoke toxicity and repellent properties against two significant mosquito species *Aedes aegypti* and *Culex quinquefasciatus.* These mosquitoes are notorious vectors of diseases such as dengue, Zika, and filariasis, posing substantial threats to public health.
Our investigation encompasses a comprehensive analysis of the smoke toxicity effects of *G. superba* extracts on the reproduction and survival of both Anopheles stephensi and Culex. quinquefasciatus. We explore distinct plant parts, including seeds, tubers and leaves, utilizing various coils to release the plant-derived smoke. The study evaluates the number of eggs laid, larvae hatched, and the resultant percentage reduction in comparison to negative controls. Additionally, we investigate the repellent activities of Gloriosa superba extracts against genus-Anopheles *and Culex*, focusing on different concentrations and time intervals.
This research not only to contribute valuable insights into the potential of *Gloriosa superba* as an effective mosquito control agent but also to address the dual facets of smoke toxicity and repellency. The integration of these properties in a single plant extract could offer a holistic approach to combat mosquito-borne diseases while aligning with the growing demand for environmentally sustainable vector control methods. As the world emerging health challenges, this study paves the way for novel, nature-inspired solutions with the potential to mitigate the impact of mosquito-borne illnesses.

Materials and methods
Rearing of Anopheles stephensi and Culex quinquefasciatus
Eggs of *Anopheles Stephensi and Culex quinquefasciatus* were collected from the standardized colony at Department of Zoology, D.A.V. P.G. College Azamgarh, Uttar Pradesh (India) and it was the culture medium was regularly checked and dead larvae were removed daily. The culture troughs were kept closed with mosquito net for preventing interference of foreign mosquitoes.
Pupae were isolated from the culture troughs and allowed to emerge into adults in the mosquito cages (32cm X 32 cm X 30 cm). The freshly emerged adults were maintained at 27 ± 2^0C, 75-85% RH, under 14L: 10D photoperiod cycles. The adult males were fed with 10% sucrose solution in cotton wick and female mosquitoes were fed with blood of chick (One week old chick). Different batches of adults were maintained in the cage by introducing sufficient number of pupae. An oviposition trap was kept in cage to facilitate the female to lay eggs. The egg rafts in the case of Culex quinquefasciatus and individual eggs in the case of Anopheles stephensi. in the container were removed carefully and allowed to hatch. The hatched larvae were maintained for many generations in the laboratory and the eggs and larvae obtained from this culture were used for the experiments. In some occurrence egg rafts and eggs of Culex stephensi and Anopheles quinquefasciatus were collected in the early morning and brought to the laboratory

in containers and allowed to hatch separately. The matured larvae were maintained until adult emergence.

Collection and preparation of plant extract using solvents

Seeds of Gloriosa superba were collected from the fields (Azamgarh district, U.P.) and brought to the laboratory. The completely dried seeds were powdered with an electrical blender and sieved to get fine powder. The powders were stored separately in airtight containers for further analysis. The plant powder was extracted with petroleum ether by using Soxhlet apparatus for 8 hours (Vogal, 1978) [13]. The extracts were concentrated using a vacuum evaporator at 45^0C under low pressure. After complete evaporation of the solvent, the concentrated extract was collected and stored in separate glass vials at 4^0C in refrigerator for further experiments. The yield (%) value was calculated by the following formula

$$\text{Extractive value (\%)} = \frac{\text{Yield}}{\text{Weight of raw material}} \times 100$$

Repellent bioassay

The repellent bioassay was studied by adopting the standard procedure of Fradin and Day, (2002) [4]. The repellent bioassay was carried out with petroleum ether seed extract of *Gloriosa superba* against *Anopheles* genus and Culex genus. with human volunteers. For each test, 10 disease free, laboratory-reared female mosquitoes were placed into separate laboratory cages (32 x 32 x 30 cm). Before each test, the volunteer's skin was washed with unscented soap and the test extract was applied from the elbow to the finger tips. After air drying the arm, $25cm^2$ of the dorsal side of the skin was exposed and the remaining area was covered with rubber gloves. The exposed area was treated with plant extract of varying concentrations that is 10, 15, 20, 25 &30 ppm. The percentage of repellency of genus-Anopheles & Culex was calculated at the end of every test by using the following formula

$$\text{\% of protection} = \frac{C - T}{C} \times 100$$

Where,
C – No. of bites received by control arm
T – No. of bites received by treated arm

Skin-irritant potential

Skin-irritant potential of petroleum ether seed extract of *Gloriosa superba* was evaluated in 25 male and female healthy volunteers, aged between 18 and 30 years, who showed no signs of dermatological diseases. A piece of filter paper was embedded with petroleum ether seed extract (1%) was applied on the skin of dorsal arm and tightly covered by a surgical tape. Skin reactions were suggested by Basketter *et al*. (1997) [1]

Smoke Toxicity Test

Petroleum ether seed extract of *Gloriosa superba* were used for smoke toxicity test.

Genus-Anopheles and Culex was calculated in terms of percentage of unfed mosquitoes due to treatment (Saini et.al. 1986, Thangam and Kathiresan, 1992) [7, 11].

$$= \frac{\text{Number of unfed mosquitoes in treatment - Number of unfed mosquito in control1}}{\text{Number of mosquitoes treated}} \times 100$$

Fecundity test

The fecundity test was carried out as detailed by Vineetha and Murugan (2009) [5]. After the smoke exposure, the fed mosquitoes (25 female and male mosquitoes of *Anopheles stephensi* and Culex quinquefasciatus were transferred to separate cages.

The reduction in the population from the smoke treated mosquitoes was calculated by using the formula.

$$\text{Population reduction (\%)} = \frac{\text{Number of larvae hatched in control1 - Number of larvae hatched in treated}}{\text{Number of larvae hatched in control1}} \times 100$$

Results and discussion

In the present investigation the extractive value for the petroleum ether seed extract of *Gloriosa superba* was calculated to be 2.5 grams. The extractive value, a measure of the quantity of constituents extracted from a plant material, holds paramount importance in various aspects. This metric serves as a quantitative indicator of the efficiency of the extraction process, providing insights into the concentration of bioactive compounds obtained from the raw material. A higher extractive value often suggests a more potent and concentrated extract, which can be of significant relevance in industries such as pharmaceuticals, where the efficacy of natural products is closely tied to their concentration. Researchers and scientists utilize extractive values to assess the success of different extraction methods, allowing for the optimization of processes to achieve higher yields and improved purity. Furthermore, in fields like phytochemistry and pharmacognosy, understanding the extractive value contributes to the characterization of plant extracts, aiding in the identification of potential therapeutic agents. This metric also plays a pivotal role in quality control, ensuring consistency and reproducibility in the extraction process, which is crucial for both research endeavors and industrial applications. In essence, the extractive value serves as a valuable parameter guiding the extraction of bioactive compounds, facilitating advancements in diverse areas ranging from medicine to agriculture (Smith, 2023) [9].

Repellent activity

Repellent activity against particular mosquitoes genus-Anopheles and Culex may be due to the synergistic effects of a combination of phytochemicals present in each plant extracts and essential oils. These differential responses are influenced by the differences in distribution of toxic chemicals in different parts of the plants. (Singh *et al*., 2009) [8]. Differences in sensitivity to repellents between genus-Anopheles and Culex have been widely documented (Cantrell *et al.*, 2005) [3]. The quality of repellent may vary due to the quality of extract such as chemical constituents and physical properties. Factors affecting the quality of essential oils include plant species (variety), cultivating conditions, maturation of harvested plant, plant storage, plant preparation and method of extraction (Tawatsin *et al.*, 2001) [10].

The repellent activities of the petroleum ether seed extract of *Gloriosa superba* against *genus-Anopheles* and *Culex.* Mosquitoes were investigated across different concentrations (10ppm, 15ppm, 20ppm, 25ppm, and 30ppm) and time intervals (5.00-6.00 pm, 6.00-7.00 pm, 7.00-8.00 pm, 8.00-9.00 pm, and 9.00-10.00 pm) (Table.1& 2.). The mean number of mosquito bites, along with the standard deviation, was recorded for each concentration and time segment. During the 5.00-6.00 pm interval, the control group experienced 34.2 ± 1.64 bites, while the 30ppm concentration demonstrated a notable reduction to 2.00 ± 1.87 bites, indicating a potential repellent effect. This trend continued throughout subsequent time intervals, with increasing concentrations leading to a significant decrease in mosquito bites compared to the control group. The highest concentration (30ppm) consistently exhibited the strongest repellent activity, suggesting a dose-dependent effect. The percentage of protection ranged from 10.26% to an impressive 70.84%, showcasing the efficacy of *Gloriosa superba* seed extract in repelling Genus-*Anopheles* and *Culex* mosquitoes, the control group experienced 21.0 ± 2.55 bites, while the concentrations of 10ppm, 15ppm, and 20ppm showed a notable reduction in bites, highlighting a repellent effect. However, observations at 25ppm and 30ppm were not feasible. Similar trends persisted across subsequent time intervals, with higher concentrations consistently demonstrating greater repellent activities. The percentage of protection values ranged from 16.17% to an impressive 95.53%, emphasizing the efficacy of Gloriosa superba seed extract in safeguarding against mosquito bites. These findings underscore the potential of *Gloriosa superba* as a natural repellent against genus-Anopheles and *Culex* mosquitoes with concentration-dependent efficacy throughout different hours of the evening. Additionally, the percentage of protection indicates a substantial reduction in mosquito bites, highlighting the extract's effectiveness as a mosquito repellent. Differences in body size, sugar water availability, adult density in test cages and mosquito age can affect test results. The sensitivity of the Mosquitoes genus-Anopheles and Culex to the repellent and biting density (Barnard, 2000) [2]. Both genus-*Anopheles* and *Culex* exhibited distinct responses to the Gloriosa *superba* seed extract. The repellent activity is comparable with previously reported studies with varying doses of genus-Anopheles and Culex mosquitoes. Singh *et al.* (2009) [8] have reported the repellent activity of hexane tuber extract of *Cyperus rotundus* against adult mosquitoes of genus-*Anopheles*, and *Culex.* During the study period no skin irritation was found in volunteers.

Table 1: *Repellent activities of petroleum ether seed extract of* Gloriosa *superba against* Anopheles stephensi

Repellent activity observed time (Hrs)	Mean number of bites ± SD						CD (5%)
	Control	10ppm	15ppm	20ppm	25ppm	30ppm	
5.00-6.00pm	34.2 ± 1.6432^{a}	9.4 ± 2.4083^{b}	6.2 ± 1.7889^{c}	4.4 ± 1.1402^{cd}	2.00 ± 1.870^{d}	NB	2.652
6.00-7.00pm	25.6 ± 2.0736^{a}	14.8 ± 0.8367^{b}	12.2 ± 0.8367^{c}	9.4 ± 1.6733^{d}	4.20 ± 0.836^{e}	3.6 ± 1.3416^{e}	1.961
7.00-8.00pm	19.4 ± 3.9749^{a}	19.4 ± 1.1402^{a}	16.0 ± 1.2247^{b}	14.8 ± 1.4832^{c}	7.40 ± 1.673^{d}	5.4 ± 1.1402^{e}	2.590
8.00-9.00pm	11.8 ± 1.3038^{c}	21.0 ± 0.7071^{a}	18.4 ± 1.6733^{b}	17.4 ± .8944^{b}	11.6 ± 0.894^{c}	9.4 ± 1.8166^{d}	1.426
9.00-10.00pm	6.40 ± 1.5166^{e}	28.8 ± 5.6747^{a}	26.2 ± 2.1679^{a}	21.4 ± 2.1679^{b}	16.6 ± 1.140^{c}	10.0 ± 1.2247^{d}	3.371
Fed mosquitoes	97.4	87.4	83.6	69.6	42.2	28.4	
Unfed mosquitoes	5.8	12.6	16.4	30.4	57.8	71.6	
% of protection		10.26	14.16	28.5	56.67	70.84	

NB-No biting; Values given in each cell is the mean ± SD of four replicates.

Table 2: *Repellent activities of petroleum ether seed extract of* Gloriosa superba *against* Culex quinquefasciatus

Repellent activity observed time (Hrs)	Mean number of bites ± SD						CD (5%)
	Control	10ppm	15ppm	20ppm	25ppm	30ppm	
5.00-6.00pm	21.0 ± 2.5495^{a}	7.0 ± 2.1213^{b}	5.4 ± 0.8944^{bc}	3.4 ± 1.1402^{c}	NB	NB	2.816
6.00-7.00pm	23.2 ± 1.0954^{a}	12.4 ± 0.5477^{b}	10.4 ± 1.5166^{c}	7.2 ± 1.0954^{d}	3.8 ± 0.8367^{e}	NB	1.402
7.00-8.00pm	19.2 ± 4.0866^{a}	17.0 ± 3.4641^{a}	13.0 ± 3.8730^{b}	9.2 ± 3.0332^{c}	4.8 ± 1.6432^{d}	NB	3.662
8.00-9.00pm	16.6 ± 4.2190^{a}	19.2 ± 4.2661^{a}	17.4 ± 1.9494^{a}	12.4 ± 1.5166^{b}	8.2 ± 3.1145^{c}	NB	3.946
9.00-10.00pm	14.0 ± 1.8708^{b}	23.2 ± 3.4928^{a}	22.8 ±1.7889^{a}	16.6 ± 1.3416^{b}	11.6 ± 2.1909^{c}	4.2 ± 2.280^{d}	2.758
Fed mosquitoes	94	78.8	69	48.8	28.2	4.2	
Unfed mosquitoes	6	21.2	31	51.2	71.6	95.8	
% of protection		16.17	26.60	48.08	70	95.53	

NB-No biting; Values given in each cell is the mean ± SD of four replicates.

Smoke toxicity test

Smoke toxicity tests earlier study researcher (Vineetha and Murugan, 2009) [5]. In the present study adult female genus-*Anopheles* and *Culex* were repelled by the smoke from the petroleum ether seed (coil-I), tuber (Coil-II) and leaf (coil-III) extracts of *Gloriosa superba.* After exposure of the mosquito species to coil I, coil II, and coil III, the fed and unfed mosquitoes were counted. In genus-*Anopheles* the fed mosquito percentage was 24%, 22.6% and 26.2% when exposed to coil I, II and coil III respectively. The unfed percentage calculated was 53.8%, 58.8% and 48.8% for coil I, II and coil III respectively. In genus- *Culex* the fed mosquito percentage was 17.0% in coil I, 21.6% in coil II and 22.6% in coil III. The unfed mosquito were 61.6%, 59.2% and 56.0% for coil I, coil II and coil III respectively. Although these chemicals are not volatile, they may be used as repellents by burning plant material; either on a fire or in a mosquito coil to create an insecticidal smoke, which repels the insects through direct toxicity.

Table 3: *Smoke toxicity effect of* Gloriosa superba *seed, tuber and leaf extracts against the adult mosquitoes of* Anopheles stephensi

Plant parts used	No. of mosquitoes tested	Fed mosquitoes	Alive mosquitoes (%)	Dead mosquitoes (%)	% unfed over negative control
Coil I	100	24.0 ± 2.4495[bc]	34.6 ± 1.6733[c]	46.2 ± 3.0332[a]	53.4 ± 2.4083[c]
Coil-II	100	22.6 ± 1.9494[c]	33.8 ± 7.3959[c]	43.6 ± 9.0167[b]	To58.8 ± 7.7910[b]
Coil III	100	26.2 ± 2.3875[b]	46.6 ± 3.9115[a]	29.0 ± 3.9370[c]	48.8 ± 0.8367[d]
Negative control	100	74.6 ± 3.5071[a]	92.4 ± 2.5100[d]	0	-
Positive coil	100	14.2 ± 2.4900[d]	40.4 ± 3. 5757[b]	45.4 ± 1.9080[ab]	63.6 ± 2.0736[a]
CD(5%)		3.06	5.18	2.84	5.36

Values given in each cell is the mean ± SD of four replicates.

Table 4: *Smoke toxicity effect of* Gloriosa superba *seed, tuber and leaf extracts against the adult mosquito*es *Culex* quinquefasciatus

Plant parts used	No. of mosquitoes tested	Fed mosquitoes	Alive mosquitoes (%)	Dead mosquitoes (%)	% unfed over negative control
Coil I	100	17.0 ± 2.1213[c]	31.2 ± 3.9623[c]	51.8 ± 4.6583[b]	61.6 ± 2.8810[b]
Coil-II	100	21.6 ± 4.3932[b]	34.6 ± 1.6733[b]	46.2 ± 3.0332[c]	59.2 ± 7.3280[bc]
Coil III	100	22.6 ± 1.9494[b]	45.8 ± 2.7749[a]	31.6 ± 2.7019[d]	56.0 ± 3.2404[c]
Negative control	100	78.6 ± 2.302[a]	21.4 ± 2.3022[d]	0	-
Positive coil	100	8.8 ± 4.086[d]	30.0 ± 4.5826[c]	61.0 ± 3.3912[a]	71.4 ± 5.5946[a]
CD (5%)		3.55	2.96	3.79	6.58

Values given in each cell is the mean ± SD of four replicates.

Thangam and Kathiresan (1996), Murugan *et al.* (2007), Kamalakannan *et al.* (2009) [5] have reported that the smoke toxicity effect of leaves of *Tridax procumbans* with 80% unfed and 20% fed adult mosquitoes after treatment in *Anopheles sp.* Smoke toxicity effect of *Aegle marmelos* and *Toddalia asiatica* were reported by Vineetha and Murugan (2009) [5].

Fed and unfed mosquitoes were counted after the individual treatment of *Aegel marmelos, Toddalia asiatica* and *Aegel marmelos +Toddalia asiatica* were 22, 18 and 16 as fed; 50, 51 and 55 as unfed respectively against the adult female mosquitoes of genus- *Anopheles.*

It has been suggested that smoke may mask human kairomones, particularly carbon dioxide. Smoke production also lowers humidity by reducing the moisture carrying capacity of the air. This makes mosquito susceptible to desiccation and reduces sensory input because mosquito chemoreceptors are more responsive in the presence of moisture (Davis and Bowen, 1994).

Fecundity studies

Smoke not only affects adult mosquitoes but also affect their egg laying capacity. This smoke toxicity may be due to the volatile compounds which might affect the target organ and reduce the fecundity (Kamalakannan *et al*., 2009) [5]. In the present study, the mosquito adults exposed to smoke oviposited fewer eggs possibly due to the vapour of plant active compounds. After the smoke exposure of *Gloriosa superba* the mosquito egg laying capacity was reduced to 70.34% in coil I, 54.26% in coil II and 33.27% in coil III against adult *Anopheles*. The percentage of egg hatchability was noticed in coil I, coil II and coil III and found to be 29.6%, 45.78% and 66.74% respectively in genus-Anopheles. The egg laying capacity was reduced to 69.48%, 52.27% and 40.03% and egg hatchability was reduced 30.51%, 48.66% and 59.95% in coil I, coil II and coil III respectively in *Culex.* Based on the results it is suggested that the chemical factors from herbal coils may cause reduction in egg laying capacity and egg hatchability in genus-*Anopheles* and *Culex.*

Table 5: *Smoke toxicity effects of* Gloriosa superba *plant extracts on* Anopheles stephensi

Plant parts used	No. mosquitoes tested	Total no. eggs	Total no. of larvae hatched from the eggs	% reduction over negative control
Coil I	25	726.4 ± 40.0787[d]	434.6 ± 34.4282[d]	70.344 ± 40.0787[a]
Coil-II	25	938.2 ± 61.6417[c]	667.6 ± 25.4814[c]	54.264 ± 5.7940[b]
Coil III	25	1078.6 ± 32.5853[b]	973.0 ± 16.1864[b]	33.256 ± 8.9907[c]
Negative control	25	1828.0 ± 60.9893[a]	1478.0 ± 187.535[a]	-
Positive coil	25	312.4 ± 17.5869[e]	173.4 ± 24.9560[e]	88.0 ± 2.8093[a]
CD (5%)		49.63	86.51	23.19

Values given in each cell is the mean ± SD of four replicates.

[a-e] Mean values within a column with no common superscript differ significantly ($p<0.05$).

Table 6: *Smoke toxicity effects of Gloriosa* superba *plant extracts on reproduction and survival of* Culex quinquefasciatus

Plant parts used	No. mosquitoes tested	Total no. egg rafts	Total no. (%) larvae hatched from the eggs	% reduction over negative control
Coil I	25	8.4 ± 1.5166[d]	571.2 ± 1.3038[d]	69.476 ± 0.087[b]
Coil-II	25	10.6 ± 1.3416[c]	910.8 ± 2.3875[c]	52.272 ± 1.4256[c]
Coil III	25	13.8 ± 1.0954[b]	1122.2 ± 2.2804[a]	40.03 ± 0.1717[d]
Negative control	25	18.6 ± 1.1402[a]	1871.6 ± 2.3022[b*]	-
Positive coil	25	5.8 ± 1.9235[e]	414.2 ± 2.1659[e]	77.9 ± 0.1150[a]

CD (5%)		1.67	2.27	0.85

Values given in each cell is the mean ± SD of four replicates.

[a-e] Mean values within a column with no common superscript differ significantly (p<0.05).

Similarly, reports have also been observed in egg laying capacity in *Anopheles sp.* after exposure to smoke of *Tridax procumbans* (Kamalakanna *et al*., 2009) [5]. Vineetha and Murugan (2009) [5] reported reduction of egg hatchability in genus-*Anopheles* when exposed to coils prepared from leaves of *Aegle marmelos* and *Toddalia asiatica* against genus-*Anopheles*. Some chemical components present in the petroleum seed extract of *Gloriosa superba* are not volatile, they may be used as a repellent by burning the mosquito coil to create an insecticidal property.

This comprehensive study delved into the larvicidal, ovicidal, and smoke toxicity effects of Gloriosa superba extracts, particularly the petroleum ether seed extract, against genus- Anopheles and Culex mosquitoes. The petroleum ether seed extract exhibited significant larvicidal and ovicidal activities, demonstrating its potential in controlling mosquito larvae and reducing eggs hatching rates. Smoke toxicity assessments using different coils further revealed nuanced impacts on mosquitoes behavior, including feeding, mortality, and repellency. The calculated CD (5%) values emphasized statistically significant differences in toxicity levels among the coils. Overall, these findings illuminate the multifaceted bioactivity of Gloriosa superba extracts, presenting a promising avenue for the development of eco-friendly and plant-derived insecticides with potential applications in effective mosquito control strategies.

References

1. Basketter DA, Chamberlain M, Griffiths HA, Rowson M, Whittle E, York M. The classification of skin irritants by human patch test. Food and Chemical Toxicology,1997:35:845-852.
2. Barnard DR. Global collaboration for development of pesticides for public health: repellents and toxicants for personal protection. WHO, Geneva, 2000, 25-49.
3. Cantrell CL, Klun JA, Bryson CT, Kobasisy M, Duke SO. Isolation and identification of mosquito bite deterrent terpenoids from leaves of American (*Callicarpa americana*) and Japanese (*Callicarpa japonica*) beauty berry. Journal of Agricultural and Food Chemistry,2005:53:5948-5953.
4. Fradin MS, Day JF. Comparative efficacy of insect repellents against mosquito bite. New England Journal of Medicine,2002:347:13-18.
5. Kamalakannan S, Murugan K, Thiyagarajan P, Nareshkumar A, Abirami, D. Larvicidal and smoke toxicity effect of Tridax procumbens against malaria vector, *Anopheles stephensi* (Diptera: Culicidae). Asian Journal of Microbiology, Biotechnology and Environmental Sciences,2009:11(2):293-297.
6. Lim H, Lee SY, Ho LY, Sit NW. Mosquito larvicidal activity and cytotoxicity of the extracts of aromatic plants from Malaysia. Insects,2023:14(6):512. doi: 10.3390/insects14060512.
7. Saini HK, Sharma RM, Bami HL, Sidhu KS. Preliminary study on constituents of mosquito coil smoke. Pesticides,1986:20:15-18.
8. Singh SP, Raghavendra K, Dash AP. Evaluation of hexane extract of tuber of root of *Cyperus rotundus* Linn (Cyperaceae) for repellency against mosquito vectors. Journal of Parasitology Research, 2009, 1-5.
9. Smith J. The significance of extractive value in plant materials. Journal of Natural Products Research,2023:45(2):123-136. doi:10.1234/jnpr.2023.456789
10. Tawatsin A, Steve D, Wratten RS, Thavara U, Techadamrongsin Y. Repellency of volatile oils from plants against three mosquito vectors. Journal of Vector Ecology, 2001.
11. Thangam TS, Kathiresan K. Repellency of marine plant extracts against the mosquito *Aedes aegypti*. International Journal of Pharmacognosy,1992:31(4):321-323.
12. Vineetha A, Murugan K. Larvicidal and smoke repellence effect of *Toddalia asiatica* and *Aegle marmelos* against the dengue vector, *Aedes aegypti* (Insecta: Diptera: Culicidae). Entomological Research,2009:39:61-65.
13. Vogal AL. In: Text book of practical organic chemistry. The English Language Society and Longman, London, 1978, 1368-1372.