LARVICIDAL ACTIVITY OF AQUEOUS LEAF EXTRACT OF *Sedum lineare* AGAINST *Culex* sp.

Atharva Charjan
Government Vidarbha Institute of Science and Humanities, Amravati, Maharashtra, India
Vaibhao Thakare
Government Vidarbha Institute of Science and Humanities, Amravati, Maharashtra, India
Anju Khedkar
Vidyabharati Mahavidyalaya, Amravati

Abstract
Insecticide resistance in mosquito vectors underscores the need for sustainable but reliable larvicides. Therefore, this work examined the larvicidal activity of aqueous leaf extract of Sedum lineare (Crassulaceae) against Culex species, which is vector of lymphatic filariasis. The leaf extracts were prepared with Soxhlet extraction with distilled water and tested in solutions of concentrations ranging from 125-500 mg/10ml, using the World Health Organization's (WHO) methods of testing the larvicides. The extract demonstrated a significant larvicidal activity, with an LC50 of 350 mg/10ml after 24 hours.. Running in parallel were the results clearly show potential eco-friendly larvicides from Sedum lineare. This is certainly worthy of additional field trials or environmental toxicity studies to non-target organisms. Culex larvae exposed to aqueous extracts of Sedum lineare gave behavioral responses to various toxic reactions such as erratic movements; air gulping with prolonged exposure resulted in dullness, inability to swim. These results suggest that Sedum lineare may be a potentially valuable source of insecticide and may well play a more prominent role in mosquito control programs in the future. Compared to known plant larvicides such as Annona squamosa and Piper nigrum, Sedum lineare leaf extracts demonstrate competitive potency. Given the availability of Sedum lineare and its production in India, this study highlights their potential as a eco-friendly larvicide. Further exploration of active compounds, synergistic effects with other botanicals, and field applicability is recommended to enhance its practical utility in integrated vector management.

Introduction

Culex sp. is a principal vector of lymphatic filariasis that afflicts millions in the tropics of which India is a part (WHO, 2023). The current use of synthetic larvicides based on organophosphates for controlling *Culex* larvae encounters problems like resistance development and environmental toxicity (Rawani et al., 2017). Biologically sourced plant-derived larvicides containing several phytochemicals, including flavonoids and phenolic acids, offer a method of control which is biodegradable, sustained, and is an attractive cost-effective alternative (Kamaraj et al., 2010). The use of single plant aqueous extract has not been addressed for a sustainable solution for mosquito control, while combinations of plant extracts have been investigated and discussed in the area of chemical synergism (Govindarajan, 2011).

Sedum lineare is a succulent species of plant native to Asia and widespread in India that has a totally high (low) flavonoid-leaf content that could be of relevance for its potential insect-repellent properties (Kim et al., 2015). To the authors' knowledge, no study has been reported that has conducted a study of the larvicidal activity of aqueous extracts of *Sedum* species against any *Culex* species. This study then focused only on the study of the larvicidal efficacy of aqueous *Sedum lineare* leaf extract against *Culex* sp. larvae via Soxhlet extraction, which was an environmentally safe method as it required no organic solvents.

Material and Methods

Plant Material Collection: Fresh *Sedum lineare* leaves were collected from the botanical gardens from Amravati Region, leaves were identified by a botanist, washed with distilled water, and sun and air-dried to prevent contamination.

Scientific Classification:
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Rosales
Family: Crassulaceae
Genus: *Sedum*
Species: *lineare*

Extract Preparation: Dried *Sedum lineare* leaf powder (50 g) was subjected to Soxhlet extraction with 300 mL of distilled water for 12 hours at 100°C. The extract was filtered through Whatman No. 1 filter paper, concentrated using a rotary

evaporator at 60°C, and lyophilized to obtain a dry powder. The yield was ~10% (w/w). The extract was stored at 4°C in airtight containers until use. The distilled water was added to the powder in the proportion of approximately 125 mg, 250 mg, 375 mg and 500 mg per 10 mL of water to from solution of 300 ml each. For preparation of solution, considered amount of powder keep packed in muslin cloth pouch and dip into required warm water for 24 hour. Then pouch removed and formed solution used for experiment.

Mosquito Rearing: *Culex species* larvae were obtained from the nearby areas in the Amravati district from stagnant and still contaminated water and were found to be abundant in the form of second and third instar larvae. These larvae were maintained at 25-30 ^{0}C, with relative humidity 60-70% under a photoperiod of (12h: 12h) following standard operating procedures for mosquito maintenance (WHO, 1975). The larvae were free of exposure to pathogens, insecticides or repellents and were maintained in beaker by providing dog biscuit or yeast powder.

Larvicidal Bioassay: The experiment adhered to WHO guidelines (2005). Larvae were transferred to 250 mL glass beakers containing 200 mL of test solution at each concentration. Three replicates were conducted per concentration, alongside a control (distilled water only). Larvae were exposed for 24 hours, with no additional food provided. Mortality was assessed by probing with a needle; larvae showing no movement were counted as dead. Observations were recorded at 0, 6, 12, 18 and 24 hours to monitor progression. LC50 value was identified on the basis of graph.

Result and Discussion

Table 1: Mortality of *Culex* larvae exposed to aqueous extract of *Sedum lineare* leaves for different interval of time

Concentration Mg/10ml	Total Respondent	Mortality			
		6 Hours	12 Hours	18 Hours	24 Hours
000	30	00	00	00	00
125	30	00	00	00	00
250	30	00	00	00	03
375	30	00	00	06	17
500	30	00	09	18	26

Figure 1: Mortality of *Culex* larvae exposed to aqueous extract of *Sedum lineare* leaves for different interval of time

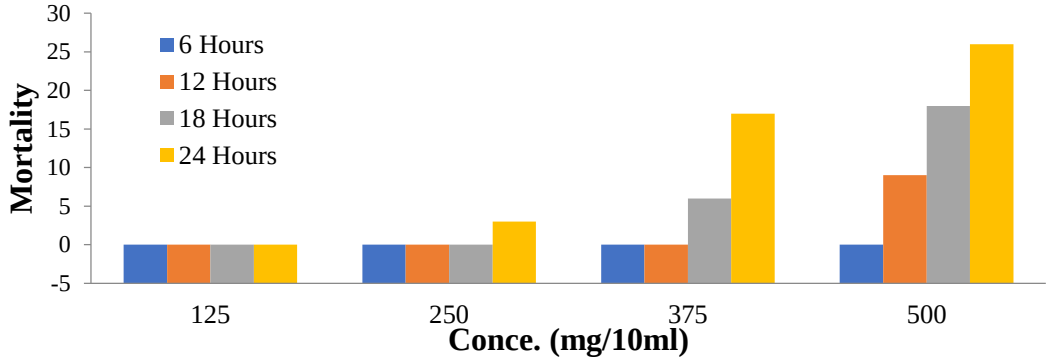

Table 2: Behavioural responses by *Culex* larvae exposed to extracts

Behaviors	Exposure Time																				
	1 hr					6 hrs				12 hrs				18 hrs				24 hrs			
	Concentrations																				
	0	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Erratic	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	-	-	-
Air Gulping	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-
Dullness	-	-	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	+	+	+	+
Loss of reflex	-	-	-	+	+	-	+	+	+	-	+	+	+	-	-	-	+	-	-	-	+
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+

: + Present/ - Absent
*: 0 Control; A 125 mg/10ml; B 250 mg/10ml; C 375 mg/10ml; D 500 mg/10ml

Figure 2: Mortality Analysis for LC_{50} for 24 hours

The powered potential of *Sedum lineare* leaf extract was determined to be larvicidal against *Culex* mosquito larvae and was exposed to treatment doses of 125, 250, 375 and 500 mg/10 ml for 6, 12, 18 and 24 hours. The assessments focused on observations of behavioural changes and mortality, the primary comparisons were made to determine the efficacy of the extract.

Behavioural responses:-

There were behavioural responses that varied due to treatment dose concentrations and exposure times in the laboratory experiment. Erratic movements were noted at all concentrations and times and was more pronounced at the higher concentrations. Air gulping was a sign of respiratory distress and was evident between 1 and 6 hours; more in the groups that were exposed to 250, 375 and 500 mg/10 ml, although it ceased after a time which suggested the larvae may have been seriously suffocating or experiencing physiological stresses. Dullness was an exact description, as a reduced activity made it clear that after 6 hours, particularly in the 375 and 500 mg/10 ml treatments, there was dullness that became more severe over time. Loss of reflex was not noted in the lower concentrations, and it was not noted until 12 hours and thereafter for the 375 and 500 mg/10 ml treatments. Discoloration was a sign of internal damage or decay and was only first noted at 18 and 24 hours for the larvae for the 500 mg/10 ml treatments.

The mortality findings demonstrated a clear dose- and time-dependent relationship. No mortality was recorded in the control or 125 or 250 mg/10 ml all exposure durations. At 375 mg/10 ml, 6 larvae were dead by 18 hours and increased to 12 by 24 hours. The highest concentration (500 mg/10 ml) showed a greater amount of mortality; with 9 larvae dead at 12 hours and increasing to 18 at 18 hours, and 100% mortality (30 larvae) by 24 hours. A bar graph showing the same trends further confirmed that larval mortality was minor at the lower dose concentrations, but a steep increase in mortality was noted at 375 and 500 mg/10 ml where larvae began exhibiting related lethargy and mortality, especially after 12 hrs as shown by both the tabulated mortality and behavioural data. The LC50 value for the leaf extract for 24 hours was approximately 350 mg/10 ml, which is the concentration that kills 50% of larvae in that time period.

Studies showed that *Sedum lineare* leaf extracts are strongly larvicidal against members of the *Culex* species, and they may be a suitable alternative to conventional, synthetic larvicides. Its larvicidal effect is equal or greater to previously used botanicals such as *Annona squamosa* and *Ocimum sanctum*, making it a formidable competitor in the same space. The larvicidal efficacy is comparable to other aqueous plant extracts, such as *Carica papaya* seed extract and *Solanum nigrum* leaf extract (Rawani et al., 2017). Kaempferol and quercetin likely disrupt larval metabolism by inhibiting enzymes like acetylcholinesterase, as observed in studies with Vitex species (Kannathasan et al., 2007).

The use of Soxhlet extraction with distilled water ensures an eco-friendly approach, avoiding toxic solvents, as specified. However, the LC50 is higher than solvent-based extracts, such as the methanol extract of *Ipomoea cairica*, due to the lower solubility of flavonoids in water (Lallawmawma et al., 2015). Using aqueous extracts has a practical side as aqueous solvents are universally used (and often a greater safety assessment than organic solvents, with hexane and methanol being less safe alternatives) because of the generally safe nature of the solvent in use. This study is the first to report larvicidal activity of *Sedum lineare* against *Culex* sp, leveraging its abundance in India and non-toxic profile (Kim et al., 2015). Compared to other plants like *Ficus benghalensis* or *Mangifera indica*, *Sedum lineare* offers moderate efficacy, ideal for integrated vector management (Govindarajan, 2010; George & Rajendran, 2015). The moderate LC50 reflects the trade-off of using aqueous extracts, which prioritize environmental safety over potency. Limitations include the need for field trials to assess efficacy in natural settings and toxicity studies on non-target organisms, such as *Chironomous circumdatus* or *Diplonichus annulatum* (Gupta, 2024). Future research could explore nano-formulations or combinations with other aqueous extracts to lower the LC50 and enhance practical applications. *Sedum lineare* leaf extract possesses a very strong and potential larvicidal activity; at a use rate of 500 mg/10 ml, the 3rd and 4th instars of *Culex* exhibited drastic behavioural arrestment and dead larvae. The compound is only a candidate to use for an effective approach to mosquito abatement because of its economic factor and environmentally friendly nature that could be considered for a permissible leave on the mosquito abatement program schedule.

Conclusion

In brief summarizing the obtained results, it was cleared that the valuation of the aqueous leaf extract of *Sedum lineare* leaves against larvae of *Culex* sp. in the laboratory showed that it was larvicidal to the mosquito species. These results suggest that *Sedum lineare* may be a potentially

valuable source of insecticide and may well play a more prominent role in mosquito control programs in the future. Further research into its specific mode of action and its lethal concentration on non target animals are recommended.

References

1. George, A., & Rajendran, S. (2015). Larvicidal activity of *Mangifera indica* leaf extracts against *Aedes aegypti*. *Journal of Vector Borne Diseases*, 52(3), 238–242. https://pubmed.ncbi.nlm.nih.gov/26418665/
2. Govindarajan, M. (2010). Larvicidal efficacy of *Ficus benghalensis* L. plant leaf extracts against *Culex quinquefasciatus* Say, *Aedes aegypti* L. and *Anopheles stephensi* Liston (Diptera: Culicidae). *European Review for Medical and Pharmacological Sciences*, 14(2), 107–111. https://pubmed.ncbi.nlm.nih.gov/20329571/
3. Govindarajan, M. (2011). Evaluation of *Andrographis paniculata* Burm.f. (Family: Acanthaceae) extracts against *Culex quinquefasciatus* (Say.) and *Aedes aegypti* (Linn.) (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine*, 4(3), 176–181. https://doi.org/10.1016/S1995-7645(11)60064-3
4. Gupta, J. K. (2024). Larvicidal activity of *Dregea volubilis* and *Bombax malabaricum* leaf extracts against the filarial vector *Culex quinquefasciatus*. *Asian Pacific Journal of Tropical Medicine*. https://www.researchgate.net/publication/384873294
5. Kamaraj, C., Rahuman, A. A., Bagavan, A., Elango, G., Zahir, A. A., & Santhoshkumar, T. (2010). Larvicidal efficacy of medicinal plant extracts against *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). *Tropical Biomedicine*, 27(2), 211–219. https://pubmed.ncbi.nlm.nih.gov/20962718/
6. Kannathasan, K., Senthilkumar, A., & Venkatesalu, V. (2007). Differential larvicidal efficacy of four species of *Vitex* against *Culex quinquefasciatus* larvae. *Parasitology Research*, 101(6), 1721–1723. https://pubmed.ncbi.nlm.nih.gov/17805567/
7. Kim, J. H., Lee, S. H., & Park, I. K. (2015). Fumigant activity of *Sedum sarmentosum* extract against stored-product insects. *Journal of Stored Products Research*, 61, 34–38. https://doi.org/10.1016/j.jspr.2015.01.004
8. Lallawmawma, H., Sathishkumar, G., Sarathbabu, S., Ghatak, S., Sivaramakrishnan, S., Gurusubramanian, G., & Kumar, N. S. (2015). Synthesis of silver and gold nanoparticles using *Jasminum nervosum* leaf extract and its larvicidal activity against filarial and arboviral vector *Culex quinquefasciatus* Say (Diptera: Culicidae). *Environmental Science and Pollution Research*, 22(22), 17753–17768. https://pubmed.ncbi.nlm.nih.gov/26154045/
9. Rawani, A., Ray, A. S., Ghosh, A., Sakar, M., & Chandra, G. (2017). Larvicidal activity of phytosteroid compounds from leaf extract of *Solanum nigrum* against *Culex vishnui* group and *Anopheles subpictus*. *BMC Research Notes*, 10(1), 135. https://pubmed.ncbi.nlm.nih.gov/28330500/
10. World Health Organization. (2005). Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/2005.13. https://www.who.int/publications/i/item/WHO-CDS-WHOPES-GCDPP-2005.13
11. World Health Organization. (2023). Lymphatic filariasis fact sheet. https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis