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Assessing the Adulticidal Efficacy of *Tylophora indica* and *Butea monosperma* Plant Extracts Against *Aedes albopictus* Mosquitoes

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Abstract: The global spread of *Aedes albopictus*, known as the Asian tiger mosquito, to 86 new countries has raised concerns about vector-borne disease transmission. The dispersal of these mosquitoes is facilitated by international trade and climate change. Effective vector control strategies and international cooperation are essential to mitigate the risk of epidemics. In India, *Aedes* mosquitoes are widespread, and insecticide resistance is a major concern. Traditional control measures have proven ineffective against *Ae. albopictus*, necessitating the development of novel tools. Plant-derived compounds have shown promise as natural pesticides. This study investigates the adulticidal efficacy of crude methanolic leaf extracts of *Tylophora indica* (*Ti*-MLE) and *Butea monosperma* (*Bm*-MLE) against *Ae. albopictus*. Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, and terpenoids in both extracts, with a higher abundance in *T. indica.* The adulticidal bioassay results demonstrated that both extracts exhibited pesticidal efficacy against the mosquitoes in a concentration-dependent manner. For *Ti*-MLE, the LC_{50} and LC_{90} values were found to be 223.43 ppm and 421.64 ppm, respectively, indicating the concentration required to cause mortality in 50% and 90% of the tested mosquitoes. For *Bm*-MLE, the corresponding LC_{50} and LC_{90} values were 326.03 ppm and 594.13 ppm, respectively. *Ti*-MLE showed higher mortality percentages and lower LC_{50} and LC_{90} values compared to *Bm*-MLE, indicating the superior efficacy of *Ti*-MLE.

Keywords: *Aedes albopictus*, Asian tiger mosquito, Vector control strategies, Insecticide resistance, Plant-derived compounds, Adulticidal activity, *Tylophora indica, Butea monosperma*

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Introduction

The global distribution of *Aedes albopictus*, commonly known as the Asian tiger mosquito, has expanded to 86 new countries through various dispersal pathways, with maritime sea transport and ground vehicles being the predominant methods of introduction. Notably, the trade of used tires and lucky bamboo containing *Ae. albopictus* eggs have played a significant role in dispersal (Swan *et al*., 2022). The colonization and establishment of *Ae. albopictus* in new geographical areas has been facilitated by globalization and climate change. Consequently, effective vector control strategies and international cooperation are crucial to minimize the risk of widespread epidemics caused by these formidable mosquito vectors (Lwande *et al*., 2019). The expansion of *Ae. albopictus* into new regions raises concerns about potential interactions with wildlife and zoonotic pathogens. Understanding its role as a bridge vector between wildlife and humans, particularly in natural breeding sites, blood-feeding behavior, and vector competence for arboviruses, is essential for assessing the risk of zoonotic transmission (Pereira-dos-Santos *et al*., 2020).

In India, *Aedes* mosquitoes are widespread and play a significant role in transmitting vector-borne diseases. Insecticide resistance, due to extensive use of insecticides in agriculture and public health, is a concerning issue (Meena, 2020). Insecticide resistance in *Aedes* mosquitoes poses a significant challenge to vector management efforts (Jangir and Prasad, 2022). Across various provinces in India, the highest resistance levels were observed against organochlorines (72%), followed by organophosphates (28%), pyrethroids (28%), and carbamates (16%). Monitoring the susceptibility of mosquito populations to insecticides is vital for devising alternative control strategies. The adaptability and resilience of *Ae. albopictus* enables it to rapidly adapt to urban environments and colder regions, making it a global threat. However, traditional control measures like larvicides and adulticides have proven ineffective in combatting *Ae. albopictus*, emphasizing the urgent need for the development of novel tools and surveillance methods (Kumawat *et al*., 2021).

Plant-derived compounds have garnered attention for their potential as natural pesticides against *Aedes* mosquitoes. Several studies have explored the larvicidal activity of various plant extracts, with promising results. The methanolic extract of *Pongamia pinnata* leaves exhibited the highest larval mortality, followed by the aqueous, ethanolic, hexane, and acetone extracts in descending order (Benelli *et al*., 2020). Similarly, the aqueous extracts of *Argemone mexicana* demonstrated superior effectiveness against *Ae. aegypti* larvae compared to flower and root extracts (Madhavi and Mahesh, 2023). Additionally, essential oils from Thyme, Camphor, and Eucalyptus have been studied for their larvicidal potential against *Ae. aegypti (*Shravani *et al*., 2023).

Tylophora indica, a threatened medicinal plant, has been extensively studied for its diverse pharmacological properties. The plant contains various active phytochemicals, including alkaloids, saponins, and tannins, which contribute to its medicinal potential (Nazar *et al*., 2020). Alkaloids extracted from *T. indica* exhibited structural diversity, highlighting their potential implications for the plant's medicinal properties (Ali and Bhutani, 1989). *Butea monosperma*, another versatile medicinal plant, is valued for its numerous traditional uses and pharmacological properties (Burlia and Khadeb, 2007). The ethanolic extract of *B. monosperma* leaves exhibited antidiabetic and antioxidant properties in diabetic mice, supporting its traditional use in managing type-2 diabetes (Sharma and Garg, 2009).

This study aimed to contribute to the growing body of knowledge regarding natural pesticides, focusing on the adulticidal efficacy of methanolic leaf extracts of *T. indica* and *B. monosperma* against *Ae. albopictus.*

Materials and Methods

Plant Material Collection:

T. indica and *B. monosperma* fresh leaves were obtained from Narsapur forest, Telangana State, India. They were thoroughly washed, and after that, they were dried in the shade for 15 days. The dried leaves were ground into a powder using a grinder and then kept in a tight bag until needed.

Extract Preparation:

T. indica and *B. monosperma* leaf powders weighing 100 g each were steeped in 250 ml of methanol separately for four days with frequent shaking to create the extracts. The solutions were filtered using Whatman filter paper no. 1 on the fifth day and left to evaporate for a full day with a revolving fan. After one day, semi-solid extracts- *T. indica* - Methanolic Leaf Extract (*Ti*-MLE) and *B. monosperma* - Methanolic Leaf Extract (*Bm*-MLE) were collected and stored at 4^0C in the refrigerator until use. To prepare stock solutions of 1000 ppm, 1 g of *Ti*-MLE and *Bm*-MLE were first combined with 10 ml of methanol and then 990 ml of distilled water, separately. Through the serial dilution process, different test solutions of 100, 200, 300, and 400 ppm concentrations were prepared from these stock solutions. The same solvents were used in the same proportions to create the control stock solution but without the extracts.

Phytochemical Analysis:

Several experiments were done to determine the secondary metabolites present in *Ti*-MLE and *Bm*-MLE. Using the Mayor's Test, alkaloids were identified. In this experiment, the Mayor's reagent was added, and a cream-colored precipitate was then noticed. The Alkaline Reagent Test was used to find flavonoids, which became yellow when NaOH was added. The Salkowski Test, in which concentrated H_2SO_4 was added, revealed the presence of terpenoids when a red or orange color developed. The Froth Test, which involves observing the production of froth upon strong shaking, was used to identify saponins. The Keller-Killiani Test, which entailed adding HCl and $FeCl_3$ and producing a red or violet color, was used to identify glycosides. The NaOH Test, which produces a yellow color when NaOH is added, was last used to identify phenols.

Insect Culture:

Mosquito larvae were collected from natural larval habitats in Narsapur town. *Ae. albopictus* larvae were identified using the pictorial keys provided by Darsie and Samanidou-Voyadjoglou (1997) and Rueda (2004). Identified *Ae. albopictus* larvae were separated from other larvae and reared on dog biscuits and yeast powder. The adult mosquitoes that emerged from these larvae were fed with sucrose solution soaked in cotton swabs. 2 – 3 days of adults were used in adulticidal bioassays.

Adulticidal Bioassay:

Adulticidal bioassay was carried out by making some modifications to the WHO (1981) method. Transparent plastic bottles used in the adulticidal bioassay were cleaned first with methanol, later with distilled water, and allowed to dry. 3 ml of the prepared solutions of *Ti*-MLE and *Bm*-MLE were poured into the bottles separately, along the walls of the bottles and they were allowed to spread all over the inner surfaces of the bottles by rotating them appropriately. The bottles were then allowed to dry and closed tightly with lids. 20 mosquitoes were released into each test tube. Cotton swabs soaked in sucrose solution were placed in each bottle. The number of dead larvae was recorded for one day at intervals of 1, 6, 12, and 24 h. The experiment was repeated three times. The per cent mortality was calculated, and the mortality data were adjusted using the formula proposed by Abbott (1925).

Statistical Analysis:

Using the MS Excel program, a probit analysis was performed on the collected results. For the analysis, a level of statistical significance of 0.05 was chosen. LC_{50} and LC_{90} values were computed by regression and probit analysis.

Results

The phytochemical analysis results of *Ti*-MLE and *Bm*-MLE revealed the presence of alkaloids, flavonoids, phenols, and terpenoids (Table 1) in both extracts. Saponins were present in small quantities in *Ti*-MLE but were not found in *Bm*-MLE. Alkaloids, flavonoids, terpenoids, and phenols were found to be more abundant in *Ti*-MLE than *Bm*-MLE.

Table 1: Identified secondary metabolites in *Ti*-MLE and *Bm*-MLE

Extracts	Alkaloids	Flavonoids	Saponins	Terpenoids	Phenols	Glycosides
Ti-MLE	++	++	+	+++	++	+
Bm-MLE	+	+	-	+	+	+

Absent: -; Slightly Present: +; Moderately present: ++; Heavily present: +++

Table 2: *T. indica* Methanolic leaf extracts (*Ti*-MLE) mortality percentages ± Standard Deviations against 2-3 days old adults of *Ae. albopictus*

Conc. (ppm)	***Ti*-MLE Mortality %** ± SD	**LC_{50} (LCL – UCL)**	**LC_{90} (LCL – UCL)**
Control	0 ± 0.47	223.43 (223.34-223.69)	421.64 (421.55-421.91)
100	33.33 ± 0.54		
200	43.86 ± 0.65		
300	63.16 ± 0.67		
400	85.96 ± 0.90		

LC_{50} – 50% Lethal Concentration; LC_{90} – 90% Lethal Concentration; LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

Table 3: *B. monosperma* Methanolic leaf extracts (*Bm*-MLE) mortality percentages ± Standard Deviations against 2-3 days old adults of *Ae. albopictus*

Conc. (ppm)	***Bm*-MLE Mortality %** ± SD	**LC50 (LCL – UCL)**	**LC90 (LCL – UCL)**
Control	0 ± 0.49	326.03 (325.96-326.25)	594.13 (594.06-594.34)
100	20.34 ± 0.67		
200	28.81 ± 0.52		
300	44.07 ± 0.62		
400	62.71 ± 0.83		

LC_{50} – 50% Lethal Concentration; LC_{90} – 90% Lethal Concentration; LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

The larvicidal bioassay results of the present study (Tables 2, 3; Fig. 1) indicated that both the plant extracts exhibited pesticidal efficacy against the adults of *Ae. albopictus* in a concentration-dependent manner. *Ti*-MLE exhibited the highest larval mortality percentage of 85.96 ± 0.90 at 400 ppm concentration. At 300 and 200 ppm concentrations the observed mortality percentages were 63.16 ± 0.67 and 43.86 ± 0.65, respectively. The lowest mortality was 33.33 ± 0.54 at 100 ppm concentration. Probit analysis results (Fig. 2) indicated that LC_{50} and LC_{90} values are 223.43 and 421.64 ppm, respectively.

Bm-MLE showed a lower level of pesticidal efficacy than *Ti*-MLE against *Ae. albopictus* adults. Even though *Bm*-MLE also showed moderate results. The highest mortality observed was 62.71 ± 0.83 at 400 ppm. 44.07 ± 0.62 and 28.82 ± 0.52 percentages of mortalities were observed at 300 and 200 ppm concentrations, respectively. The lowest mortality obtained was 20.34 ± 0.67 at 100 ppm concentration. Probit analysis results (Fig. 3)

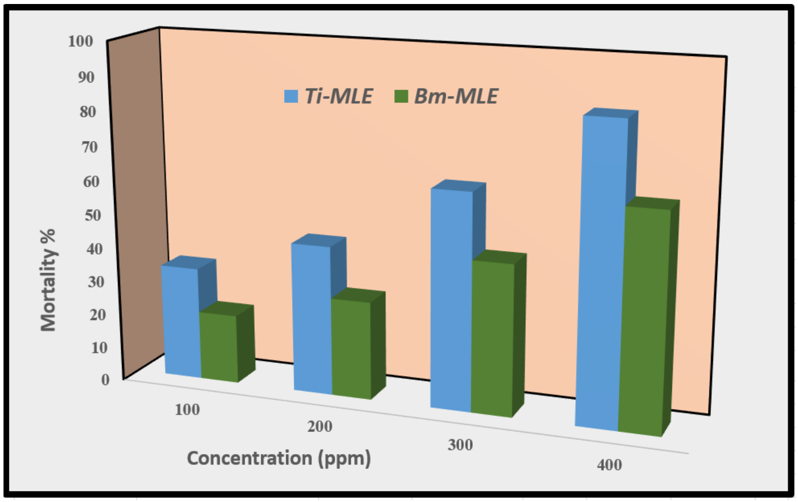

Fig. 1: Adulticidal efficacy of Methanolic Leaf Extracts of *Tylophora indica* (*Ti*-MLE) and *Butea* monosperma (*Bm*-MLE) against *Ae. albopictus* mosquitoes.

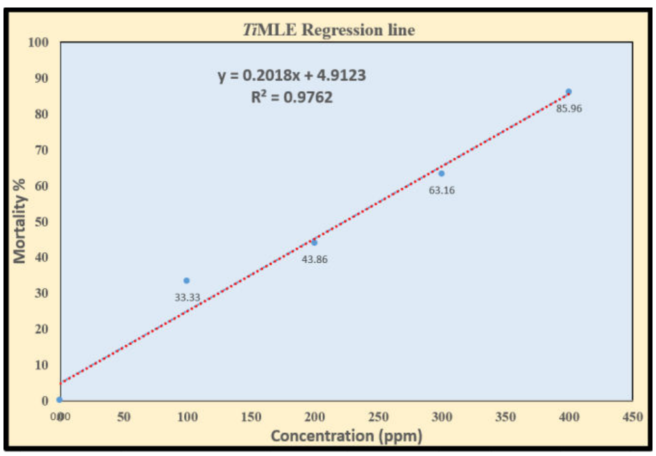

Fig. 2: Regression analysis of the Adulticidal bioassay of Methanolic Leaf Extracts of *Tylophora indica* (*Ti*-MLE) against *Ae. albopictus* mosquitoes.

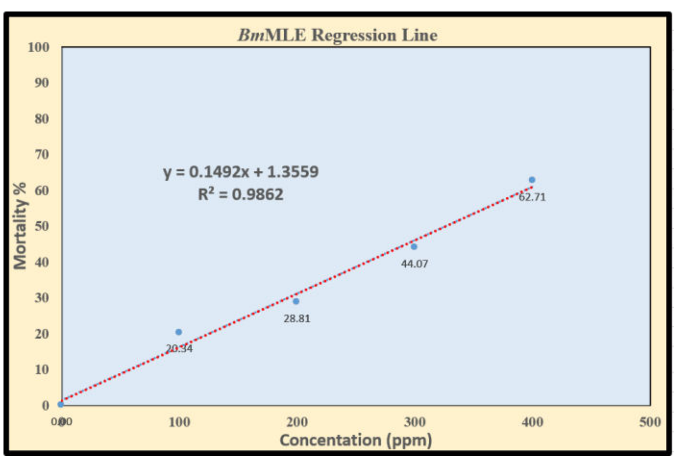

Fig. 3: Regression analysis of the adulticidal bioassay of Methanolic Leaf Extracts of *Butea* monosperma (*Bm*-MLE) against *Ae. albopictus* mosquitoes.

indicate that LC_{50} and LC_{90} values are 326.03 and 594.13 ppm, respectively.

Discussion

Phytochemical analysis of *Ti*-MLE and *Bm*-MLE in the present study confirmed the presence of bioactive compounds with pesticidal potential. Comparatively, *Ti*-MLE is rich in all the tested secondary metabolites than *Bm*-MLE. The results of the adulticidal bioassays support this difference in phytochemical compounds as *Bm*-MLE yielded less efficient results in killing the mosquitoes than *Ti*-MLE. The results of the phytochemical analysis of the present study are in line with the results of previous studies. Borkar *et al*. (2010) reported the phytochemicals present in the leaf extracts of *B. monosperma*. Gururani *et al*. (2020) revealed the different types of secondary metabolites in *T. indica* leaf extracts.

The study highlights the effectiveness of *Ti*-MLE and *Bm*-MLE in controlling *Ae. albopictus* mosquitoes. The results of the present study are in line with the previous studies. Gandhi *et al.* (2014) evaluated *Ti*-MLE for their pesticidal efficacy against *Culex quinquefasciatus* and *Aedes aegypti*. They reported LC_{50} values of 478.64 ppm for *Cx. quinquefasciatus* and 819.19 ppm for *Ae. aegypti*. A natural compound Palasonin derived from the *B. monosperma* plant demonstrated high ingestion toxicity to *Plutella xylostella* (LC_{50}: 10.72 mg L^{-1} at 48 h) and high contact toxicity to *Periplaneta americana* and *P. xylostella* (LD_{50}: 0.08 µg/adult and 0.22 µg/larva) (Fan *et al*., 2022). The presence of secondary metabolites which are proven to possess pesticidal and various biological effects might be responsible for the mortality of *Ae. albopictus* mosquitoes.

Conclusion

The study investigated the pesticidal efficacy of *Ti*-MLE and *Bm*-MLE, against *Ae. albopictus* mosquitoes. Phytochemical analysis of *Ti*-MLE and *Bm*-MLE revealed the presence of alkaloids, flavonoids, polyphenols, and terpenoids in both extracts. The adulticidal bioassay results demonstrated that both extracts exhibited pesticidal efficacy against the mosquitoes in a concentration-dependent manner. T*i*-MLE showed higher mortality percentages and lower LC_{50} and LC_{90} values compared to B*i*-MLE, indicating the superior efficacy of T*i*-MLE. The study provides valuable insights into the potential use of T*i*-MLE and B*i*-MLE as environmentally friendly and sustainable options for mosquito control. Further research could explore the mode of action of these extracts, their potential impacts on non-target organisms, and their feasibility for application in larger-scale pest management programs.

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