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SYNERGISTIC LARVICIDAL ACTION OF INDIGENOUS PLANT EXTRACTS IN COMBINATION AGAINST *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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Abstract

The most effective methods for addressing the problems of insecticide resistance and environmental pollution are expected to be phytochemicals. The current study focuses on evaluating the effectiveness of metabolites from three indigenous plants in combination (*Argemone mexicana*, *Tinospora cordifolia*, and *Prunus persica*) in controlling *Aedes aegypti* mosquito larvae. The leaf and seed of *Argemone mexicana*, *Prunus persica*, and the leaf of *Tinospora cordifolia* and two solvents (ethanol and petroleum ether) making a total 7 combination groups (A to G) were used in bioassay to determine LC_{50} and LC_{90} values. Combination group- A (ethanolic + ethanolic extracts of seed of *Prunus persica* and *Argemone mexicana*) LC_{50} and LC_{90} values of 70.79 and 169.59 ppm after 24 h post-exposure. Similarly, other combination groups- B, C, D, E, F, and G with LC_{50} values of 58.88, 74.13, 81.28, 89.12, 61.65, and 57.54 ppm, respectively, and LC_{90} values of 147.91, 173.78, 229.08, 269.15, 162.18, and 151.35 ppm, respectively, within 24 h. In combination, against the third instar larvae, all the treatments were shown great larvicidal potential ($P<0.05$). The regression equation showed a dose-dependent mortality, the mortality rate was positively correlated with the concentration. Results showed plant metabolites can be used in combination as eco-friendly insecticides for the control of dengue vectors.

Keywords**:** *Aedes aegypti*, *Argemone mexicana, Prunus persica, Tinospora cordifolia*, larvicidal action, LC_{50}, Combination.

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Introduction

Aedes aegypti is an anthropophilic and domicile mosquito, a vector of various diseases that can spread dengue virus, Zika, Chikungunya, and other viral diseases (Halder *et al.*, 2011; Ghosh *et al.*, 2012). Throughout the world, an estimated 400 million people get infected with dengue (WHO, 2021) and about ten lakh cases of dengue infection and thousands of deaths were reported only in India, in the last five years (MOHFW, 2021). The most popular technique for controlling mosquitoes is the use of synthetic chemical pesticides. Previous studies have shown that mosquitoes can become resistant to both synthetic and biological pesticides, including *Bacillus thuringiensis* (Wattal *et al.*, 1981; Tabashnik, 1994). *Aedes aegypti* mosquito has developed stronger resistance against chemical insecticides such as DDT, permethrin, cyfluthrin, and lambda-cyhalothrin (Jangir and Prasad, 2022). The use of chemical insecticides affects nontarget organisms, environmental health (Benelli, 2015), and risks of toxicity in human beings such as chromosomal abnormalities and cholinesterase inhibition in peripheral leukocytes (Sharma *et al.*, 2016).

Insecticide resistance and ineffective results have made it necessary to look for new alternative methods and the selective principle has become essential for the control of mosquitoes with less environmental pollution. Plant-based products are the most suitable alternatives to synthetic chemical insecticides (Sukumar *et al.*, 1991; Ghosh *et al.*, 2012). In this context, previous studies have reported that plant-derived bioinsecticides are biodegradable, environmentally friendly, and showed potential larvicidal action against mosquitoes (Tiwary *et al.*, 2007; Arokiyaraj *et al.*, 2013; Benelli, 2016). *Argemone mexicana* (family – Papaveraceae) a single plant has countless advantages in drug discovery with interesting pharmacological properties viz., Anti-cancer activity, antimicrobial activity, nematocidal activity, antihelminth activity, larvicidal and antifeedant action, etc (Nancy and Praveena, 2017). The leaf and seeds of this plant exhibit insecticidal action against different insects, *Culex quinquefasciatus* (Sakthivadivel *et al.*, 2012), *Tribolium castaneum* (Patil and Zambare, 2019), *Spodoptera litura fab* (Vetal and Pardeshi, 2019), and *Chrysoperla carnea* (Aragon-Sanchez *et al.*, 2020).

Tinospora cordifolia (family – Menispermaceae) is known for its massive use in the treatment of various illnesses in traditional Ayurveda. Indigenous to tropical regions of Indian subcontinents (Sinha *et al.*, 2004; Saha and Ghosh, 2012). Different parts of this plant exhibit insecticidal or larvicidal properties against different insects (Sharma *et al*., 2003; Abdullah *et al.*, 2012; Paul *et al*., 2020). *Prunus persica* (family – Rosaceae) commonly known as peaches. Peaches have a variety of health benefits, including anti-inflammatory, anti-allergic, anti-tumor, and anti-cancer qualities (Kant et al., 2018), and insecticidal or larvicidal activity (Seo and Park, 2012; Carneiro *et al*., 2021). As a result of the aforementioned characteristics, *Argemone mexicana*, *Tinospora cordifolia*, and *Prunus persica* were chosen for their combined larvicidal activity against the third instar of *Aedes aegypti*.

Material and Methodology

Collection of plant material

The leaves and seeds of *Argemone mexicana*, *Prunus persica*, and the leaf of *Tinospora cordifolia* were collected from the district Bulandshahr region (28.4070° N, 77.8498° E), Utter Pradesh, India. Selected plants are not threatened or endangered. The plant materials were taken from healthy plants and washed with distilled water. The collected materials were dried for a period of 20 days at 30^{o}C, in shade. The dried materials were powdered with help of an electric grinder and the powdered material was placed in a 500 ml sterile beaker for subsequent processing.

Extraction

Ethanol and petroleum ether were used as a solvent. Ten-gram powdered materials of each were extracted in 200 ml of solvents serially in glass Soxhlet apparatus, separately for a duration of 24 hours at the temperature of solvents boiling points (Kasiramar, 2018). After 24 hours, the remaining solvent evaporated in the water bath to make solvent-free extracts. After complete evaporation of solvents, the remaining residue was weighed and dissolved in distilled water. The extracts (2000 ppm) were stored at 4°C as the stock solution.

Collection and culture of eggs and Larvae

Aedes aegypti eggs and larvae were collected from mosquito ovitraps set on the different locations on the college campus or different locations of the district Bulandshahr, Utter Pradesh, India. Larvae were reared in the research laboratory of N.R.E.C. College, Bulandshahr. A plastic tray (20 cm X 15 cm X 5 cm) filled with tap water was used to culture the larvae. The protocol described by Kamaraj *et al*., (2009) was followed in the laboratory setting to rear the mosquito larvae at 27±2 °C, RH 75±5%, and L/D photoperiod 14:10 hours. Brewer yeast powder and dog biscuits (3:1) were provided to larvae as food. Eggs were hatched in dechlorinated water. Larvae of *Aedes aegypti* were identified with the help of a binocular microscope by following the identification key (Christophers, 1960; Rueda, 2004).

Larvicidal bioassay

Ten ml of Tween-20 (emulsifying agent) were added to the extracts of the leaf and seeds (2000 ppm stock solution). This solution was diluted with distilled water to get the required concentrations (50, 100, 200, 400, 500, and 1000 ppm). Twenty larvae were put into five replicates of a beaker containing 150 ml of extract. The larvae were not given any food during the exposure time. 5% ethanol and tween-20 dissolved in distilled water served as the control. In accordance with WHO recommendations, LC50 and LC90 values were calculated for the treatment that resulted in 100% larval mortality after 24 hours (2005).

Statistical analysis

The data obtained after 24 h exposure was subjected to probit analysis (Finney, 1971) for the calculation of LC_{50} and LC_{90} values with 95% confidence level, the regression equation by using the software program "MS Excel 2021". When required, the control mortality was corrected by using the Abbott formula (Abbott, 1925).

Table 1 : Plants selected for their larvicidal action.

S. No.	Plant species	Family	Part used
1.	*Argemone mexicana L.*	Papaveraceae	Seed and leaf
2.	*Prunus persica (Thunb.)*	Rosaceae	Seed and leaf
3.	*Tinospora cordifolia L.*	Menispermaceae	Only leaf

Table 2 : Preparation of a combination (mixture) of ethanolic and petroleum ether extracts of seed and leaf of selected plants.

S. No.	Combination groups	Combination	Ratio
1.	A	AMES+PPES	50:50
2.	B	AMPS+PPPS	50:50
3.	C	AMES+PPPS	50:50
4.	D	AMEL+PPEL+TCEL	33:33:33
5.	E	AMPL+PPPL+TCPL	33:33:33
6.	F	AMESL+PPESL+TCEL	40(50:50):40(50:50):20
7.	G	AMPSL+PPPSL+TCPL	40(50:50):40(50:50):20

Abbreviation:

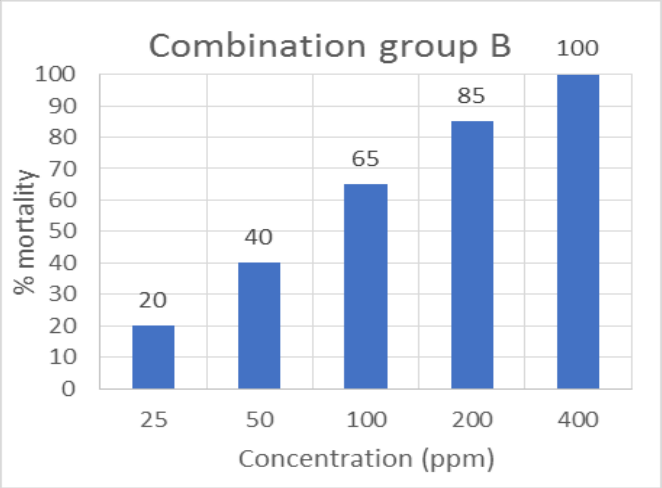
AMES - *Argemone mexicana* ethanolic extract of seed.
AMPS - *Argemone mexicana* petroleum ether extract of seed.
AMEL - *Argemone mexicana* ethanolic extract of the leaf.
TCEL - *Tinospora cordifolia* ethanolic extract of the leaf.
PPESL - *Prunus persica* ethanolic extract of seed and leaf.
AMPSL - *Argemone mexicana* petroleum ether extract of seed and leaf.
PPES - *Prunus persica* ethanolic extracts of seed.
PPPS - *Prunus persica* petroleum ether extract of seed.
PPEL - *Prunus persica* ethanolic extract of the leaf.
AMESL - *Argemone mexicana* ethanolic extract of seed and leaf.
PPPSL -*Prunus persica* petroleum ether extract of seed and leaf.
TCPL - *Tinospora cordifolia* petroleum ether extract of leaf.

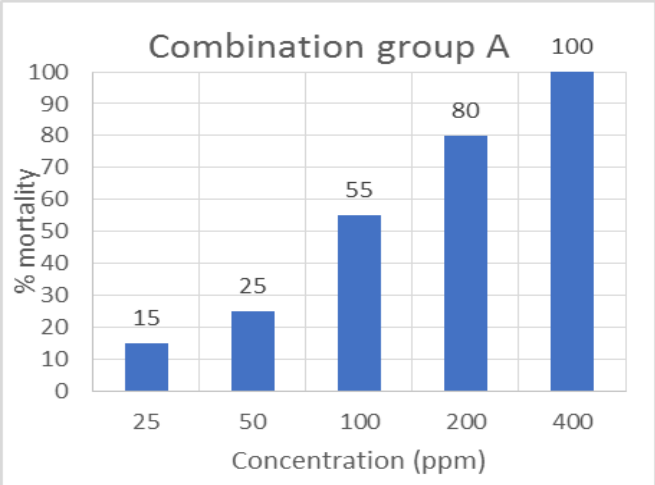
Table 3 : Larvicidal potential of extracts in combination against the third instar of *Aedes aegypti*.

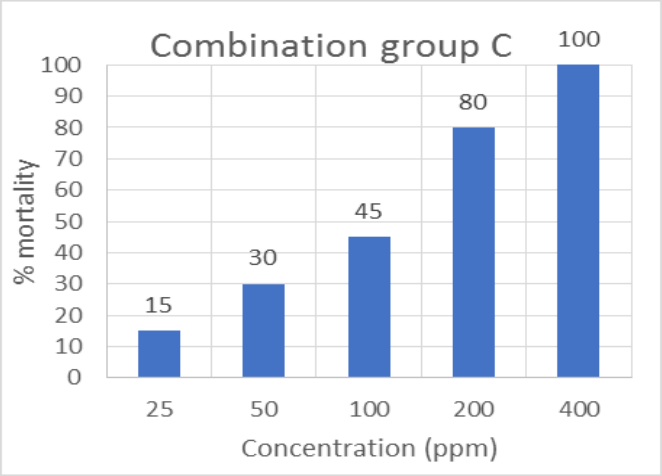
Mosquito species	Combination group	Conc. (PPM)	Mortality (%)	LC50 (PPM)	LC_{90} (PPM)	95% confidence interval		Regression equation	R^2	P-value (P<0.05)
			24 h	24 h	24 h	Lower bound	Upper bound			
Aedes aegypti	A	400	100±0.0	70.79	169.59	2.0621	4.7406	Y=3.4014x-1.2924	0.92	0.002
		Control	00±00							
	B	400	100±0.0	58.88	147.91	2.0338	4.2930	Y=3.1635x-0.6089	0.93	0.001
		Control	00±00							
	C	400	100±0.0	74.13	173.78	1.9795	4.9774	Y=3.4785x-1.5273	0.91	0.002
		Control	00±00							
	D	500	100±00	81.28	229.08	1.3601	4.3266	Y=2.8434x-0.4497	0.87	0.005
		Control	00±00							
	E	500	100±00	89.12	269.15	0.9480	4.5048	Y=2.7264x-0.3354	0.81	0.01
		Control	00±00							
	F	400	100±00	61.65	162.18	1.4545	4.7176	Y=3.0861x-0.545	0.87	0.006
		Control	00±00							
	G	400	100±00	57.54	151.35	1.5824	4.4508	Y=3.0167x-0.3064	0.89	0.004
		Control	00±00							

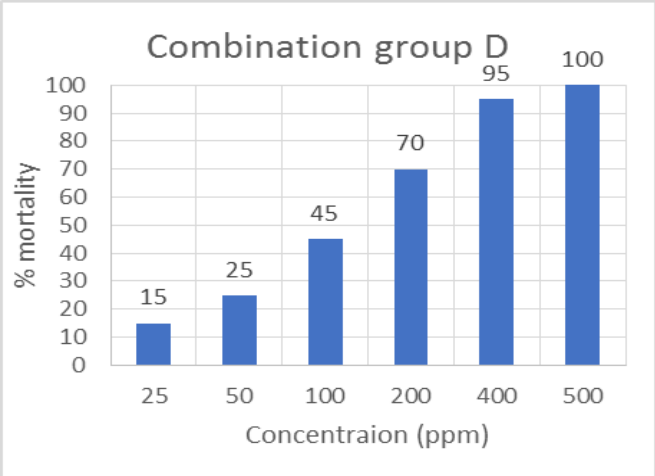
Result

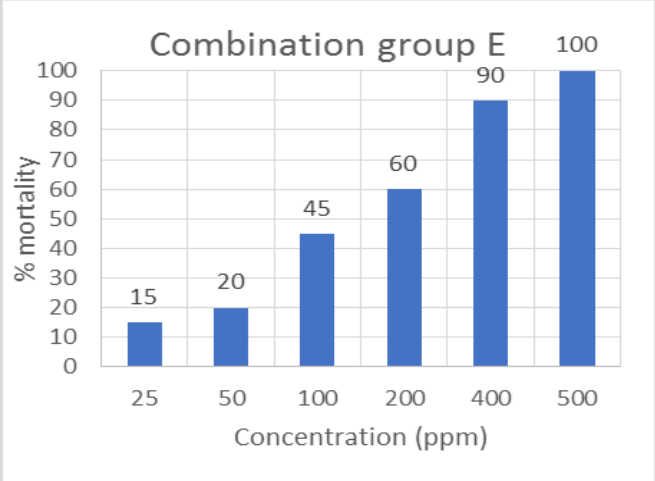
Table 1 shows that the leaf and seed of *Argemone mexicana*, *Prunus persica*, and the leaf of *Tinospora cordifolia*, and two solvents (ethanol and petroleum ether) were used to make a total 7 combination groups (table 2-3) were used in bioassay to determine LC_{50} and LC_{90} values. Combination group- A, ethanolic + ethanolic extracts of seed of *Argemone mexicana* and *Prunus persica*, (50:50) with LC_{50} and LC_{90} values of 70.79 and 169.59 ppm after 24 h post-exposure. Similarly, other combination groups- B, C, D, E, F, and G with LC_{50} values of 58.88, 74.13, 81.28, 89.12, 61.65, and 57.54 ppm, respectively, and LC_{90} values of 147.91, 173.78, 229.08, 269.15, 162.18, and 151.35 ppm, respectively, within 24 h post-exposure. In combination, all the extracts displayed great larvicidal activity (P<0.05) against the third instar of the dengue vector. No larval mortality was seen in the control groups.

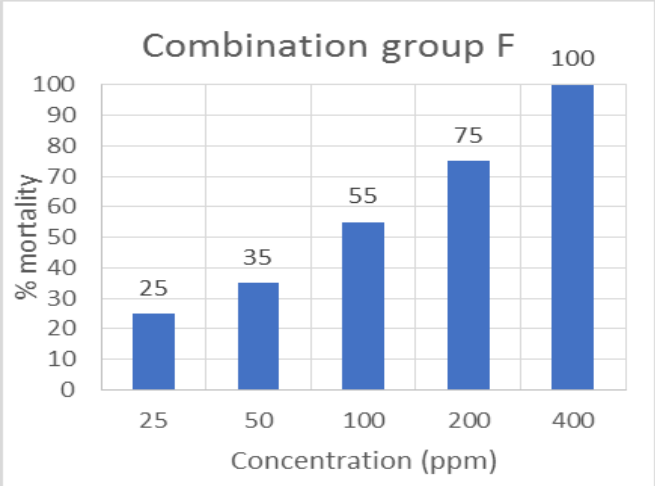


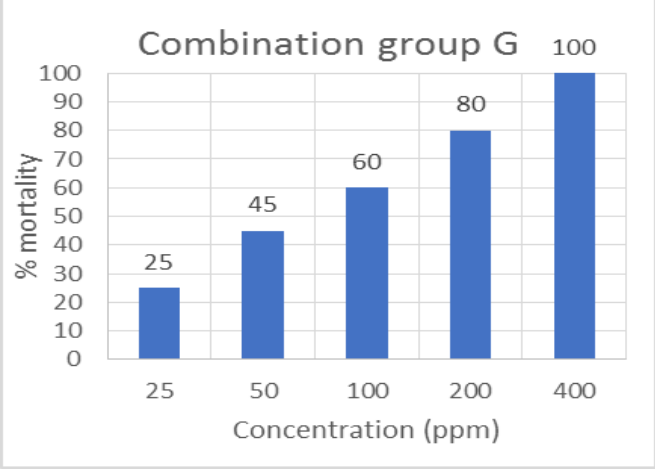












Discussion

Plant extracts exhibit a variety of biological responses in mosquitoes as a result of a complex mixture of phytochemicals that may be cooperating to cause such a reaction. Plant-derived bioinsecticides seldom result in the development of insect resistance due to the coordinated action of biomolecules (Maurya *et al.*, 2012).

In previous studies, the different solvents extract of seed and leaf extracts of *Argemone mexicana*, *Prunus persica*, and *Tinospora cordifolia* have been shown to have larvicidal activity against different insects. However, there are few studies on the combination of plant extracts as a larvicide against mosquitoes. Individually, the leaf and seed extracts of *Argemone mexicana* LC_{50} range between 250-400 and 100-150 ppm, respectively (Ruiz-Guerrero *et al.*, 2015). In the present study, in combination with leaf and seed extracts of *Prunus persica*, the larvicidal activity of *Argemone mexicana* was enhanced. Ethanolic extracts of *Argemone mexicana* seeds in combination with ethanolic extract of seed of *Prunus persica* with LC_{50} values of 70.79 ppm and Petroleum ether extract of *Argemone mexicana* seed in combination with petroleum ether extract of *Prunus persica* with LC_{50} values of 58.88 ppm (table 3).

Individually, the ethanolic extract of Tinospora cordifolia's leaf demonstrated larvicidal activity against *Aedes aegypti* with an LC50 value of 162.88 ppm, and larvicidal activity of a combination of *Tinospora cordifolia* (ethanol) + *Andrographis paniculate* (methanol) against *Aedes aegypti* with LC50 values of 113.20 ppm (Paul *et al.*, 2020). But in the present investigation, the ethanolic extract of the leaf of *Tinospora cordifolia*, *Argemone mexicana* and *Prunus persica* exhibits great larvicidal action in combination against the third instar of *Aedes aegypti* larvae with LC values of 81.28 ppm within 24 h, the larvicidal activity was statistically significant ($P<0.05$).

For their toxicities, hundreds of plant species have been tested against mosquitoes (Rehman *et al.*, 2014). As expected, the compounds extracted using different solvents, from diverse parts (stem, root, bark, leaf, flower, etc.) of plants were tested on the larvae and adult mosquitoes, and there was a variation in the larvicidal action (Brahmachari *et al.*, 2013). Impact of phytochemicals extracted from the stem of *Argemone mexicana* altered behavior and morphological modification in *Aedes* larvae (Warikoo and Kumar, 2014). Chakraborty *et al.* (2021) reported that crude and ethanolic extracts of *Asparagus setaceus* demonstrate efficient larvicidal activity against *Aedes aegypti* larvae within 24 h. The chloroform, methanolic, and petroleum ether extracts of four plant species, *L. camara*, *N. oleander*, *H. suaveolens*, and *T. stans* demonstrated significant larvicidal potential on *Aedes aegypti* and *Culex quinquefasciatus*, individually or in combination (Hari and Mathew, 2018). Furthermore, the toxic action of the plant *Maerua siamensis* on the *Aedes aegypti* larvae, with LC_{50} value of 71.14 ppm within 24 h (Nobsathian *et al.*, 2018) and *Clitoria ternatea* has been shown to have larvicidal activity on *Aedes aegypti*, with LC_{50} and LC_{90} values of 1056 ppm and 2491 ppm, respectively (Ravindran *et al.*, 2020). *Vitex ovata* at a concentration of 5000 ppm and 10000 ppm achieved 76% and 84% *Aedes aegypti* larval mortality (Aziz *et al.*, 2021). Methanolic extract of *Aegle marmelos* and *Coleus aromaticus* tested against the different larval stages and pupa of *Aedes aegypti*,

showed significant larvicidal action in 24 h (Dass *et al*., 2022). *Rhanterium epapposum* ethanolic, acetone and aqueous extracts of leaf showed 98%, 72%, and 30% larval mortality at a higher concentration of 50000 ppm (Asiry, 2022).

Conclusion

In conclusion, the plant *Argemone mexicana*, *Prunus persica* and *Tinospora cordifolia* are here studied. The ethanolic and petroleum ether extracts of leaf and seed in combination enhanced the larvicidal potential of selected plants and showed significant larvicidal activity against the third instar of *Aedes aegypti* larvae. At the present time, the protection of the environment is taken seriously. It is important to promote environmentally friendly insecticides that kill enough target species to keep insect populations below the threshold level.

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Conflict of interest

The authors hereby declare that there are no conflicts of interest with respect to the publication of this paper.

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