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Synergistic effect of *Andrographis echioides* and *Cadaba trifoliata* leaf extracts against larvae of dengue mosquito *Aedes aegypti* L

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ABSTRACT

Objective: The larvicidal potential of *Andrographis echioides* and *Cadaba trifoliata* leaf extract was tested separately against larvae of *Aedes aegypti* and also tested mixture of these two plant extracts for synergistic effect against the larvae. **Methods:** Pulverized leaves of *A. echioides* and *C. trifoliata* were subjected separately to soxhlet extraction using organic solvent of ethanol. These two plant extracts were examined separately against 4th instar larvae of *A.aegypti* with gradually increasing concentration i.e. from 50 to 250mg/l using WHO protocol. To observe the synergistic effect, extracts of these two plants were mixed at different concentrations and tested against the larva. The 24h LC_{50} values of individual plant extract and mixed extracts were determined following probit analysis. **Results:** *A. echioides* extract shows more lethal effect than *C. trifoliata* extract towards larvae of *A.aegypti* with LC_{50} values of 108.3 and 123.4 mg/l, respectively, whereas , synergistic larvicidal effect was found to be even more effective than the plant extract tested separately in terms of LC_{50} being 68.3 mg/l. **Conclusions:** From the results, it is evident that synergistic effect of *A. echioides* and *C. trifoliata* can be considered as a more powerful arsenal for the control of *A.aegypti* than the usage of these two extract separately.

1. Introduction

Insect transmitted diseases are important health problems in tropical and sub tropical regions. Insects form the largest class of the animal kingdom and include nearly 80% of known animal species. In the order diptera is included medically and veterinary important insects as the flies and the mosquitoes [1]. Currently much interest is focused on the control of mosquitoes that are the major vector for the transmission of malaria, filaria, dengue and several diseases. In this context, *Aedes aegypti* represents a particularly important target for control since it is responsible for the transmission of dengue fever, dengue hemorrhagic fever, yellow fever and chikungunya. World wide, 40–80 million individuals are infected with dengue virus each year [2]. On the other hand, there is no vaccine for dengue fever [1]. Besides their role in disease transmission, mosquito nuisance can adversely affect real estate value, tourism and related business interests [3]. In India, *A.aegypti* is found in all parts, its distribution and abundance being strongly influenced by the dense population and increasing deforestation.

The current global strategy for the control of vector borne diseases is based on vector control, achieved through larvicidal (control of larvae), adulticidal (control of adults), and bite prevention (applications of repellents and bed nets). Larvicidal is more effective than adulticidal, because it is target specific, that is, treatments are applied to relatively small areas where larvae are concentrated, and hence the overall usage of pesticides for mosquito control is reduced [3]. Chemical control remains a main component of integrated vector management. The development of resistance to chemical insecticides in mosquitoes, together with enhanced concerns about the environmental impact of most synthetic products [4] has prompted the search for alternative natural products. The use of plant extracts for mosquito control save the environment from synthetic chemicals and also gives more employment opportunities to Self Help Women Group in Tamil Nadu. The present experimental plants *A. echioides* and *C. trifoliata* belongs

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to the family of acanthaceae and capparaceae, respectively. *A. echioides* widely distributed in the tropical India and Srilanka. The leaf juice of this plant is used as remedy for fevers [5]. *C. trifoliata* is an unarmed branched shrub. Leaves of this plant possess antirheumatic, anthelmintic and antibacterial properties [6]. In the present study, we have evaluated the synergistic effect of *A. echioides* and *C. trifoliata* against larvae of *A.aegypti*.

2. Materials and methods

2.1. Mosquito rearing

The larvae of *A.aegypti* collected from the field were maintained at 70-85% relative humidity, 27±20 °C temperature and 14:10 light and dark photoperiod cycle. The larvae were fed on a powdered mixture of dog biscuits and yeast tablets in the ratio of 3:1. The emerged adults were fed with rabbit blood with 10% glucose solution in a mosquito cage.

2.2. Extraction and stock solution

Leaves of *A. echioides* and *C. trifoliata* were collected locally, shade dried at room temperature, powdered coarsely and each powdered plant material was extracted with ethanol for eight hours in the soxlet apparatus (300 ml of ethanol for 100g leaf powder) separately. The liquid extracts were concentrated in a rotary vacuum evaporator to yield green crude extracts. Stock solution (1000ppm) of each plant crude extract was prepared by dissolving 250 mg of the crude extract in 5ml of ethanol and making it to 250ml by mixing distilled water in a standard flask.

2.3. Larvicidal activity

The larvicidal activity of the *A. echioides* and *C. trifoliata* leaf extracts was evaluated against larvae of *A.aegypti* by using the standard method [7]. The test concentrations were made by diluting the known volume of stock solution of the extract with 250ml water to obtain the desired test concentrations (50,100,150,200 and 250 mg/l). Control was set up simultaneously with 249ml filtered tap water, to which 1ml ethanol was added. Twenty five early 4th instar larvae were introduced to each of the test solutions as well as control. Small, unhealthy or damaged larvae were removed and replaced. Five replicates were set up for each concentration and control. Each test was run three times on different days. The larval mortality was recorded after 24h of post treatment. Moribund larvae were counted and added to dead larvae for calculating percentage mortality.

2.4. Synergistic activity

In synergistic larvicidal activity, another set of the concentrations were prepared from two stock solutions. Five different concentrations of *A. echioides* at 50, 100, 150, 200 and 250 ppm (125 ml of each concentration) were mixed with 125 ml of 50,100,150,200, and 250 ppm of *C. trifoliata*, respectively. The final volume of each mixed test concentration was 250 ml. The larvicidal activity of the mixed extracts was evaluated as per the above mentioned method in larvicidal activity. The synergistic factor (SF) was calculated using the following formula of Kalyanasundaram and Babu [8] with slight modification.

SF = LC_{50} value of the individual plant extract

LC_{50} value of the combined plant extracts

A value more than one, confirm synergism; the greater the value, the higher the synergistic activity.

2.5. Statistical analysis

The observed percent mortality was adjusted for the control mortality, using Abbot's formula [9], and then subjected to propit analysis [10] for find out lethal concentrations of 50% and 90% mortality. One way variance analysis (ANOVA) was performed followed by Tukey's test using SPSS software. P value less than 0.05 were considered to indicate statistical significance.

3. Results

In the search for an eco-friendly pesticide, researchers have considered pesticides of biological origin, and the replacement of chemical pesticides with biopesticides as a generally acceptable one. The yield of crude extract obtained from 400gm of *A. echioides* and *C. trifoliata* leaf powder were 35 and 40g, respectively. The crude extracts were subjected to laboratory bioassay studies against larvae of *A. aegypti*. Data on the concentration-mortality response of *A. echioides* and *C. trifoliata* are given in Table1. The lethal concentrations of *A. echioides* and *C. trifoliata* leaf extracts at LC_{50} and LC_{90} were determined as 108.3, 223.4 and 123.4, 245.6, respectively. A positive correlation was observed between the crude extract concentration and the per cent mortality, the rate of mortality being directly proportional to concentration. However, 1.4% mortality was observed with control, which ensured that the solvent used to dissolve the extract did not contribute to the overall mortality during the bioassay. It is clear from the data obtained that *A. echioides* extract was more toxic than *C. trifoliata* extract to larvae of *A. aegypti*. After exposure to the crude extracts, the treated larvae exhibited restlessness, sluggishness, tremors and convulsions followed by paralysis at the bottom of the bowl. The Chi-square values of 2.7 and 2.2 were significant at $P<$ 0.05level.

Table 1 also reveals the results of synergistic larvicidal effect of *A. echioides* and *C. trifoliata* mixture. The mixture of

these extract showed a greater larvicidal effect over the *A. echioides* and *C. trifoliata* tested separately. The LC_{50} value of the mixed extract was 68.3, it was less compare to LC_{50} values of *A. echioides* and *C. trifoliata* independent extract. The synergistic value of *A. echioides* and *C. trifoliata* was 1.6 and 1.8, respectively, the synergistic values were greater than one. The results clearly indicate that the mixed extract exhibit synergistic effect against larvae of *A. aegypti*.

Table 1

Synergistic larvicidal activity of *A. echioides* and *C. trifoliata* leaf extract on 4th instar larvae of *A. aegypti*.

Plant species	Concentration (mg/l)	Mortality (%)
A. echioides	50	36.3 ±1.4a
	100	49.5 ±1.7b
	150	79.7 ±2.1d
	200	98.3±1.4e
	250	66.3±1.7c
C. trifoliata	50	24.5 ±1.4a
	100	39.3±1.4b
	150	61.7±1.9c
	200	72.7±2.3d
	250	93.5±1.7e
A. echioides + *C. trifoliata* (Synergism)	50	42.3±1.4a
	100	58.5±1.7b
	150	79.3±1.4c
	200	94.7±2.1d
	250	100±0.0e
	Control	1.4±0.3f

Each value (mean±SEM) represents mean of five replicates value. Values with different letters in a column are significantly different at $P<0.05$ level (Tukey's test).

4. Discussion

Aedes aegypti is a domiciliary mosquito, which hides in dark and closed places, aggravating its eradication. The ideal method for controlling mosquito infestation is the prevention of mosquito breeding through the larvicides. *A.aegypti* commonly used in insecticide screening trials because it is an anthropophilic species (human biter), larvae are more robust and less susceptible to insecticides and botanical extracts than *Culex and Anopheles* species, and easy to colonize in the laboratory [11]. We selected a polar solvent ethanol in the extraction of samples, because of, most of the bioactive components have been reported to be hydrophilic, at the same time ethanol is low cost, easily available in the market and ecofriendly [12] (made from sugarcane molasses). As per the previous studies by Chahad and Boof [13], they evaluated the effect of *Piper nigrum* extracts with *Culex quinquefasciatus* larvae, the extract obtained using the soxhlet method produced higher mortality and reduced lethal time than the extract obtained by maceration and other techniques. In this regard, we used soxhlet method for the extraction of plant material.

The LC_{50} values of *A. echioides* and *C. trifoliata* in the current study are significant and far superior than those reported earlier [1, 14, 15], in their studies, LC_{50} values of bark & heartwood extract of *Myracrodruon urundeuva*, leaf essential oil of *Clausena dentata* and essential oils of *Hyptis fruticosa* & *Hyptis pectinata* against *A.aegypti* is 881 & 1486, 140.2, and 502 & 366 mg/l, respectively, and they have shown a need for very high dosage of plant extracts for achieving significant mortality of larvae. The sluggish movement and peculiar coiling of treated larvae in our study seems to suggest some neural or muscular disturbance, which caused an acute lethal effect. In this study, we used 4th instar larvae, because, the earlier larvae were easily susceptible by the treatment due to the age and physiological status of the larva. Most of the previous studies highlighted on synergistic effects on agricultural pests rather than pest of medical importance. The few studies on the mosquitocidal activity of binary mixtures have investigated the combined effects of phytochemicals with insecticides or microbial control agents. To our knowledge only few report of synergistic larvicidal activity of binary mixture of two plant extracts against mosquito species was found. We found in our research that the synergistic activity of binary mixture of *A. echioides* and *C. trifoliata* only require 68.3mg/l to make 50% mortality. It was lower than LC_{50} values of *A. echioides* and *C. trifoliata* tested separately. The present synergistic results are comparable with mixture of two plants extracts *Croton caudatus* and *Tiliacora accuminata* caused 100% mortality when exposed to 75mg/l concentration after 48h [16].

As per the previous study, synergism may be due to insecticide inhibiting a larva's ability to employ detoxifying enzymes against larvicidal compounds in the plant extract. In the present study, the same above said mechanism may be involved in synergism. Individual plant extracts need high dosage for control than binary mixture of plant extracts, which makes them uneconomical for field use. This synergistic binary mixture can be incorporated into mosquito control programs, so as to avoid indiscriminate use of synthetic insecticides and to reduce dosage of individual plant extracts. Because of its significant larvicidal property and local availability as they grow abundant in the villages, it might form a new arsenal for vector management, especially in areas where mosquitoes have developed resistance to conventional insecticides, as well as practical in application with minimum care by individuals and communities. The findings of the present work suggest, products based on these plant extracts may contribute greatly to a reduction in environmental chemicalisation, and to an overall reduction of the population density of *A. aegypti*.

Conflict of interest statement

We declare that we have no conflict of interest.

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