



Extracts of *Lantana Camara* Induced Toxicity and Acetylcholinesterase Inhibition Against Rice Pest *Leptocorisa Oratorius*.

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KEYWORDS

Biopesticide, toxicity, rice pest, pest management, contact toxicity, *Lantana camara*, insecticide.

ABSTRACT: The present study aims to find the toxicity of different extracts of *Lantana camara* against the rice pest, *Leptocorisa oratorius*. It also intends to evaluate the acetylcholinesterase inhibitory property of the most active extract under study. Contact toxicity studies showed that 15% of the ethanol extract showed a high mortality rate of 92%, followed by methanol and aqueous extracts. Toxicity increased in a dose-dependent manner. Likewise, the plant ethanol extract causes 87.25% inhibition of acetylcholine esterase activity in insect physiology. It was determined that the *L.camara* plant extract has substantial insecticidal properties and acetylcholinesterase inhibitory effect against *L.oratorius*.

1. INTRODUCTION

Rice is the most economically important food crop all over the world. The rice bug *Leptocorisa oratorius* (Hemiptera: Alydidae) is a major pest in the rice fields worldwide. The nymphs and adults of the pests feeds on the developing grains resulting in partial or empty grains and account for a yield reduction ranging from 10% to total crop failure depending on degree of infestation [1]. *L.oratorius* is a major pest of economic importance [2]. Intensive use of the chemical insecticides in the rice field to control this pest destroys non target organisms and accumulation of toxic residues in the environment [3]. Aimed at the sustainable increase of rice production appropriate pest management using botanicals is essential. Researchers worldwide are in

search of eco-friendly pest control alternatives, especially of plant origin [4]. Owing to their high toxicity to targeted insects low developmental cost and being harmless for the farmer community and with little effect on the environment, the botanical insecticides are widely accepted [5]. Insecticidal activity of some Plants like *Acornus calamus* [6], *Vitex negundo*, *Azadiracta indica*, *Gnidea glauca* [7] *Jatropha curcas* have been established against *L. oratorius*. Nevertheless a very

few promising studies on plant-based biopesticides have been conducted against this pest. *L. camara* belongs to Verbenaceae family and is a notorious invasive weed reported to be native to tropical, subtropical and temperate regions. It has been reported that solvent extracts of *Lantana camara* exhibited insecticidal potential against stored grain pests [8]. This plant also demonstrates larvicidal [9], nematocidal, antifungal and antibacterial properties [10]. Numerous studies identify this plant as a potent natural insecticide with an extensive range of phytochemical profiles. Studies reported that this plant also has an enzyme-modulating effect on insect physiology. The present study is designed to evaluate the contact toxicity and acetylcholinesterase inhibitory activity of *L.camara* against *L.oratorius*.

2.OBJECTIVE

The objective of the present study is to find the insecticidal efficiency of different extracts of *Lantana camara* against major rice pest *L.oratorius* and to evaluate its acetylcholinestrerase inhibitory property on insect.



3. METHODS AND MATERIALS

Insect Rearing

Rice pests, *L.oratorius*, collected from paddy fields of Vellayani (8°28'44°N 76°59'33°E) were taken to the laboratory and identified by appropriate keys [11]. The unsexed adult insects are kept for egg-laying in an Insect Oviposition Facility. Rice seeds of good quality from the Integrated Farming System and Research Station (IFSRS), Karamana, are collected and brought to the laboratory. The seeds were soaked overnight and wrapped in a cotton cloth for germination. Seedlings of seven days are planted in a small land area with a continuous supply of water. When the seedlings produce the milky rice grains after three months, the whole facility is covered by a nylon net (1.5mm x 1m) and keeps the unsexed *L. oratorius* from the paddy field for oviposition. Eggs were collected and placed in a petri dish with wet cotton and kept in a perforated plastic container of 22 cm height and 9 cm diameter with a nylon net covered at the top for adult emergence.

Collection of Plants

The plant used in the study is *L.camara* (Lamiales:Verbenaceae), sourced from Peyad, Trivandrum district, Kerala (30'29" N 59'54" E). The identity of each plant species was confirmed by a botanist.. The plant parts were washed, shade-dried for fourteen days and ground to a fine powder. The dried, powdered plant materials were extracted with 95% ethanol, methanol and aqueous through soxhlet extraction. From 10% stock solution, working solutions of 15, 10, 5 and 3% concentrations were prepared. This trial included two control treatments, a positive control of the commercially available chemical pesticide ekalux and a negative control of distilled water with acetone.

Contact toxicity bioassay

For mortality bioassay a drop (0.25 µL) of test solution is applied topically onto the dorsal surface of the thorax of each insect using a hand micropipette. Each trail includes 15 insects. Placed the treated insects on rice seedlings or an alternate host *Echinocola* covered by perforated plastic containers with 22 cm and 9 cm width. Proper aeration and moisture were ensured by spraying water from the top of the container every 12 hr.. Recorded insect mortality after 72 hours of treatment. Mean percentage mortality was calculated

using the Abbott formula [12]. Insect body weight was also recorded. Five replicates of the experiment are taken

Enzyme assay

Ethanol extract-treated *L. oratorius* adults that survived after the toxicity experiment were washed with distilled water and the excess adhering water was removed by blotting with tissue paper. For enzyme assay, the survived insects were homogenized in ice-cold sodium phosphate buffer (20 mm, pH 7.0) by using a Teflon hand homogenizer and centrifuged at 8000 rpm for 20 minutes at 4 °C. Supernatant was used for enzyme assays. The homogenate was kept on ice until used for further assays [13]. Acetylcholinesterase (AChE) activity in the whole-body homogenates of *L.oratorios* was estimated. [14]. Donepezil was used as the standard.

Statistical Analysis

For statistical analysis, the results were presented as mean ± SE. Percentage mortality was corrected by Abbott's formula. The data for different concentrations were subjected to a Probit analysis program to determine the median lethal concentration values (LC50) and other regression parameters. The data was subjected to ANOVA to test the difference between various concentrations and controls using SPSS (16.0) software. Values with $P \geq 0.05$ were considered as statistically significant.

RESULTS

Table 1 shows the percentage adult mortality in *L.oratorius* following exposure to ethanol, methanol and aqueous extract of *L.camara* after 72 hours. *L.oratorius* showed a high mortality of 92% at 15% concentration of ethanol extract. This is followed by 81.33% and 80.53% at 15% concentration of methanol and aqueous extracts of *L. camara*, respectively. At 10% concentration, maximum mortality is shown by methanol(74.33%) extract, followed by ethanol (72%) and aqueous extract (68%) after 72 hours of exposure. Mortality at 5% concentration is recorded as 60%,62.67% and 57.34% for ethanol, methanol and aqueous extract, respectively. Lower most mortality rates of 41.33%,41.30% and 42.66% are observed in 3% concentration of ethanol, methanol and aqueous extract of *L. Camara*, respectively. Control trials show 100%



mortality. Among the three extracts, ethanol shows the lowest LC 50 value of 3.63 % (P value 0.02) followed by methanol of 3.8% (P value 0.01) and aqueous extract

of 4.07% (P value 0.01) respectively, after 72 hours of exposure.

Table 1: Mean values of percentage mortality after treating *L.oratorius* with different solvents.

Solvent	Mean±SE					
	Control(-ve)	3%	5%	10%	15%	Control(+ve)
Ethanol	0.0±0.0	41.33±0.48 ^a	60.00±.054 ^a	72.00±0.58 ^a	92.00±0.6 ^a	100±0.0 ^a
Methanol	0.0±0.0	41.30±0.58 ^a	62.67±0.67 ^a	74.33±0.54 ^a	81.33±0.75 ^b	100±0.0 ^a
Aqueous	0.0±0.0	42.66±0.60 ^a	57.34±0.75 ^b	68.00±0.37 ^b	80.53±0.37 ^b	100±0.0

*a,b,c,d indicates the different letters are significantly different from each other at 0.5% level of significance

Table 2: LC₅₀ values after treating *L.oratorius* with ethanol, methanol and aqueous extracts of *L.camara*

Solvent	LC 50(%)	X ²	Slope±SE	p
Ethanol	3.63(2.49-5.02)	9.52	2.15±0.33	0.02
Methanol	3.8(3.37-4.87)	10.27	1.52±0.19	0.01
Aqueous	4.07(3.50-4.73)	10.9	1.45±0.16	0.01

Table 3: Percent inhibition of Acetylcholinesterase activity in *L.oratorius* treated with ethanol extract of *L.camara*.

Insect	Concentration	Acetylcholinesterase (Inhibition%)
<i>Leptocorisa oratorius</i> (F=25.46,df=2,P value<0.05)	3%	23.72±0.04 ^a
	5%	62.07±0.09 ^b
	10%	76.65±0.45 ^c
	15%	87.25±0.07 ^d
	Control(-ve)	17.85±0.42 ^a
	Control(+ve)	91.85±0.03 ^d

*a,b,c,d :means sharing the same letter within each treatment is not statistically different

The percent inhibition of Acetylcholinesterase in *L.oratorius* after 72 hrs of treatment with the most effective ethanol extract is shown in table 3. Maximum acetyl cholinesterase inhibition of 86.72% was observed at a concentration of 15%, followed by 76.65%, 62.07% and 23.72% at 10, 5 and 3%, respectively. A positive control, which is a standard acetylcholinesterase inhibitor drug, shows an inhibition of 91.84 % in *L. oratorius* and a negative control, which is an insect with no treatment, shows a low inhibition percentage of

17.44%. Percent inhibition increases as the concentration of the extract increases (Table 3).

DISCUSSION

Plant extracts have a wide range of bioactive molecules that can be used as potent insecticides and can act as a promising alternative to chemical pesticide. The present study is designed to find out insecticidal potency of a weed plant *L. camara* against *L.oratorius*, a major pest in the rice field now a days. The current study observed



that *L. camara* showed very high contact toxicity at all concentrations under study. The toxicity increases as the concentration of the extract increases. The toxicity is confirmed by using ethanol, methanol and aqueous extracts. All these extracts showed very good promising results. Of these the ethanol extract at 15% concentration shows the maximum mortality of 92% after 72 hrs of treatment.

It has already been reported the indigenous traditional practice of using *L. camara* flowers along with other plant extracts against *Leptocorisa* [15]. Some previous studies suggested that a few plants like *Azadiractin indica*, *Vitex negundo*, *Andrographis paniculata*, *Azadiractin indicum*, *Citrus reticulata*, *Acornus calamus*, *Nicotiana tabacum*, *Ocimum basilicum*, *Calotropis* extracts are effective in reducing population of *L. oratorius* species in the field trails [16].

Studies shows the contact toxicity of ethanol extract of *L. camara* against many other insects, such as *Tribolium castaneum* and *Lasioderma serricornis* [10]. Some Previous studies also reported the repellent property of *L. camara* against mosquitoes [17]. It is also found that the methanol extract from leaves of *L. camara* has fumigant and contact toxicity against *Sitophilus oryzae*, *Callosobruchus chinensis* and *T. castaneum* [8]. Thus, *L. camara* extract is a very promising candidate for the development of a very potent bio pesticide against *L. oratorius*. As this plant is an invasive weed, its availability is also very high.

As the ethanol extract shows very high mortality of 92%, this extract was further selected for examining its inhibitory capacity on acetylcholinesterase in insect physiology. Acetylcholinesterase is a very prominent target candidate for the development of many available chemical insecticides. The present study showed that the ethanol extract of *L. camara* has a percentage acetylcholinesterase inhibition of 87.25%, 76.65%, 62.07% 23.72% at 15, 10, 5, 3 % concentrations. This indicates the very high acetylcholinesterase inhibitory action of this plant. The inhibitory percentage is near to the positive control Donepezil, which is a standard acetylcholinesterase inhibitory drug. Inhibition of acetylcholinesterase (AChE) by Coumaran, an active ingredient extracted

from the plant *L. camara*, has been previously reported [8]. Acetylcholinesterase inhibitory activity of essential oil from *Lantana camara* flowers is also reported [19]. Many investigations confirmed the AChE inhibiting activity of some phytochemicals present in *L. camara* [10]. These studies thus shed more light on the potential of this plant species as a naturally occurring insecticide.

A few plants had previously reported insecticidal potential against *Leptocorisa* species. The scientific studies conducted against this species of pest are very limited. This study is a very promising report that shows the high insecticidal potential of an invasive weed, *L. camara*, against *L. oratorius*. The current study also confirms the acetylcholinesterase inhibitory potential of *L. camara* on the pests' physiology.

CONCLUSION

Lantana camara plant extract showed toxicity against the rice pest *Leptocorisa oratorius*. Based on the LC50 values among the different extracts under study, the ethanol extract shows the highest mortality rate. This extract also causes a very high acetylcholinesterase inhibitory rate in insects. Additional study is recommended to find the plant's complete phytochemical profile and to find out its mode of action in the insect body. Further investigations are highly recommended as this plant has a high potential of developing into a future natural pest control candidate.

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