



Bio Efficiency of Medicinal Plant Extracts Against Rice Pest, *Leptocoris* *Oratorius* as an Alternative to Chemical Pesticides.

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ABSTRACT:

Conventional chemical pesticides cause a lot of biotic and abiotic hazardous effects, and the need for an eco-friendly alternative is vital. Bioactive compounds from plant extracts are promising candidates to be established as potential insecticides. The current study investigates the insecticidal potential of five aqueous plant extracts, including *Cassia fistula*, *Annona muricata*, *Atrocarpus heterophyllus*, *Coleus aromaticus* and *Clerodendron infatunatem* against *L.oratorius*. Qualitative analysis of the aqueous plant extracts was also examined for its phytochemical constituents. Maximum mortality was caused by *C. fistula* (77.34%) at 15% and minimum mortality was observed in *C. infatunatem* (17.33%) at 3% concentration. Various phytochemicals of high bioactivity like phenols, tannins, flavonoids, saponins, terpenoids, alkaloids, quinones and fatty acids present in the plants under study have the potential to act as insecticides against the rice pest *L.oratorius*.

1. Introduction

The rice bug, *Leptocoris* *oratorius* (Hemiptera:Alydidae) also called as Gundhi bug is a serious pest attacking rice fields in India [1], tropical and subtropical regions of the world. They feed on the foliage and flowers of leguminous and gramineous crops. Substantial infestation results in complete loss of the crop. Widespread use of chemical pesticides in the rice field to control this pest causes many adverse effects on biotic and abiotic communities. Rice, is an important crop, and research has been conducted worldwide in search of eco-friendly pest control alternatives like botanical insecticides, especially from plant extracts [2] as they ensure safety for the farmer community, are easily affordable, have low developmental cost and with minimal effect on the environment. Some studies have established the insecticidal activity of *Acornus calamus*, *Vitex negundo*, *Azadiracta indica* and *Jatropha curcas* against *L.oratorius* [3] in field trials.

To date, very few promising studies on the pertinence of plant-based biopesticides have been conducted against

this pest. Furthermore, plant-based traditional knowledge has been identified from the tribal communities, are being used against these pests for generations [4]. Additional investigation of these plants is required for their insecticidal bioactive compounds to develop environmentally safe insect control agents in future.

2. Objectives

The objective of the present study is to evaluate the insecticidal potential of five aqueous plant extracts against *L.oratorius*. The contact toxicity of *C.fistula*, *A.muricata*, *A.heterophyllus*, *C.aromaticus* and *C. infatenatum* against *L. oratorius* is investigated.

3. Methods

Insect Rearing

Rice pests, *Leptocoris* *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 [5]. 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 7 days are planted in a small land area with a continuous supply of water. When the seedlings produce the milky rice grains after 3 months, the whole facility is covered by a nylon net (1.5mm x 1m) and keeps the unsexed *Leptocorisa oratorius* from paddy field for oviposition and adult rearing.

Collection of Plants

Leaves and flowers of locally available plants are collected from Peyad, Thiruvananthapuram District, Kerala (30°29'N 59°54'E). The identity of each plant species was confirmed by a botanist.

Extraction

Fresh leaves and flowers of the plants were collected, washed, cut into pieces and shade-dried for 14 days. The dried leaves were ground to a fine powder and plant materials were extracted with distilled water using Soxhlet apparatus. The extracts were concentrated in a rotary evaporator and stored refrigerated which is used for the bioassay. Working concentrations of 15,10,5 and 3% are prepared from this stock solution in distilled water. Two controls are taken, a positive control of commercially available chemical pesticide Ekalux and a negative control of distilled water.

Phytochemical analysis

The qualitative analysis of the plant extracts was done to screen the presence of bioactive compounds like flavonoids, phenolic compounds, terpenoids, glycosides, quinones, alkaloids, fatty acids [6], steroids, tannins [7].

Topical toxicity of plant extracts

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 test involves 15 insects using five plants with four different concentrations. Five replicates of each experiment are taken. Place the treated insects on rice seedlings of milky grain stage planted in a pot (30cm) covered by perforated plastic containers with 22 cm height and 9cm diameter containing nylon net cover at the top. Place this in a water tray. Spraying water every 12 hrs from the top of the containers ensures proper

aeration and moisture. Recorded insect mortality after 72 hrs of treatment and converted them into mean mortality by Abbot's formula [8]. Insect body weight is recorded.

Statistical Analysis

The results were presented as mean ± SE. Percentage mortality was corrected by the Abbott formula. The data for different concentrations were subjected to Log Probit Model Analysis to determine the median lethal concentration values (LC 50) and other regression parameters. The data was subjected to ANOVA to test the differences in mortality between various concentrations and controls using SPSS (16.0) software. Values with $P \geq 0.05$ were considered as statistically significant.

4. Results

Results represented in Table 1 show the phytochemical screening of five pesticidal plants namely *C.fistula*, *A.muricata*, *A.heterophyllus*, *C.aromaticus* and *C.infortunatum*. *C.fistula* leaves and flowers showed the presence of phenols, tannins, flavonoids, saponins, alkaloids, glycosides, quinone and fatty acids, among this, the phenols and quinones are in high concentration. The absence of terpenoids and steroids is also noted in *C.fistula* in the present study. In *A.muricata* leaves phytochemicals tannins, saponins, terpenoids and alkaloids are observed in high concentrations along with the absence of quinones and steroids. *A.heterophyllus* showed the presence of phenols, tannins, flavonoids, saponins, terpenoids, alkaloids and glycosides. The present study reported *C.aromaticus* leaves have phenols, tannins, saponins and quinones in low concentration. *C.infortunatum* showed the presence of phenols, tannins, flavonoids, saponins and quinones in low concentrations. The absence of terpenoids, alkaloids, glycosides, fatty acids and steroids is also noted.

The percentage mortality of five different aqueous plant extracts against *L.oratorius* is presented in Table 1. Comparisons between positive and negative controls are also shown. Among the extracts, the highest mortality percentage of *L.oratorius* was observed with 77.34% of *C.fistula*, followed by *A.muricata* with 62.67%, *A.heterophyllus* with 48.56%, *C.aromaticus* with 46.67% and *C.infortunatam* with 46.34% after 72 hours.



of exposure. At 15% concentration, all plants under study shows high mortality (>45%). The LC₅₀ towards *L.oratorius* among the plant extracts were 4.16 for *C.fistula*, followed by 6.60% for *A.muricata*, 9.56% for *A.heterophyllus*, 17.51% for *C.infortunatam* and 17.78% for *C.aromaticus*. Among the plant extracts, *C.fistula* followed by *A.muricata* were very promising in killing

50% of *L.oratorius* population. *C.aromaticus* with LC₅₀ 17.78% is found to be the least efficient among the plants under study. The positive control, Ekalux, a commercially available chemical pesticide is superior to all plants, and shows a mortality of 100% and negative control distilled water with 0% mortality in all selected plant extracts.

Table 1: Phytochemical composition of aqueous extract of five selected plant species.

Plant species	Phytochemical Constituents of Five Plant Species									
	Phenol	Tannin	Flavonoid	Saponin	Terpenoid	Alkaloid	Glycoside	Quinones	Fattyacid	Steroid
<i>C.fistula</i>	high	low	low	low	t	moderate	moderate	high	low	t
<i>A.muricata</i>	moderate	h	t	high	high	high	moderate	t	low	t
<i>A.heterophyllus</i>	high	low	low	high	low	moderate	high	t	t	t
<i>C.aromaticus</i>	high	low	t	moderate	t	absent	low	low	low	t
<i>C.infortunatam</i>	low	low	low	moderate	t	absent	absent	low	t	t

Table 2: Percentage mortality of aqueous extracts of Five plant species against *Leptocorisa oratorius*

Plants	Mean ± SE					
	Control(-ve)	3%	5%	10%	15%	Control(+ve)
<i>C. fistula</i>	0.0±0.0	49.34±0.60 ^{a*}	48.00±0.37 ^a	62.67±0.50 ^b	77.34±0.50 ^c	100±0.0
<i>A. muricata</i>	0.0±0.0	37.34±0.50 ^b	46.67±0.63 ^c	58.67±0.58 ^d	62.67±0.51 ^d	100±0.0
<i>A. heterophyllus</i>	0.0±0.0	28.67±0.40 ^a	40.34±0.67 ^d	46.78±0.40 ^c	48.56±0.44 ^c	100±0.0
<i>C. aromaticus</i>	0.0±0.0	22.67±0.40 ^a	37.33±0.67 ^b	42.67±0.40 ^c	46.67±0.44 ^c	100±0.0
<i>C.infortunatam</i>	0.0±0.0	17.33±0.20 ^a	29.35±0.24 ^a	37.36±0.51 ^b	46.34±0.24 ^c	100±0.0

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

Table 3: LC₅₀ values after treating adult *Leptocorisa oratorius* with aqueous extracts of selected plant extracts.

Plants	LC 50(%)	X ²	Slope±SE	P
<i>C.fistula</i>	4.16(3.07-5.57)	6.76	1.09±0.32	0.079
<i>A.muricata</i>	6.60(4.03-7.32)	13.63	0.93±0.05	0.003
<i>A.heterophyllus</i>	9.56(6.78- 9.75)	14.44	1.34±0.37	0.006



<i>C.aromaticus</i>	17.78(13.00-18.80)	17.21	0.88±0.23	0.065
<i>C.infortunatem</i>	17.51(12.81-18.66)	17.44	0.87±0.23	0.067

5. Discussion

At all concentrations tested, aqueous extracts of *C.fistula*, *A.muricata*, *A.heterophyllus*, *C.aromaticus* and *C. infatenatum* showed remarkable toxicity against *L.oratorius*. Some previous studies suggested that a few plants like *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[9]. Laboratory studies against this pest are very much limited. All plants under this study show good insecticidal potential against *L.oratorius*. The efficiency of these plants against *L.oratorius* has not yet been reported. Even though all plants in this study exhibit insecticidal potency against *L.oratorius* adult, their toxicity differs probably due to differences in phytochemical constituents.

Plant-based insecticides contain a wide range of bioactive compounds or phytochemicals that has the latent to cause adverse effects on insect's physiology when exposed to them. *C.fistula* phytochemical analysis shows positive results for Tannins, Phenols, Saponins, Flavanoids, Terpenoids, Alkaloids, Glycosides, Quinones and fattyacids. This is in confirmation of the previous studies showing the constituents of the plant extracts [10]. Our present study shows phenols and quinones as the greater concentration in *C.fistula*. Phenols have been previously reported to have antitumour, anti-inflammatory, and anti bacterial properties which also act as an antioxidant [11]. Quinines from Cassia species show antifungal activity [12] and larvicidal [13] activity against mosquito larvae. Cassia species can be utilized as biopesticides due to their potential insecticidal properties[14]. *A.muricata* aqueous extracts show high concentrations of tannins, saponins and terpenoids. This is consistent with previous reports of constituents of aqueous extract of this plant[15].It has been reported previously that acetogenin found in the leaves of *A.muricata* shows insecticidal property and can be used to control trips, and ladybug attacks [16].Quinones and fatty acids are absent in aqueous

extracts of *A.heterophyllus* is shown to be less effective than *A.muricata* and *C.fistula*.*C.aromaticus* and *C.infortunatem* is comparatively less effective than other plants under study.All the above-mentioned plant extracts have been previously reported to have insecticidal, larvicidal and antimicrobial property.

A few plants had previously reported insecticidal potential against *Leptocorisa* species in field trial.The scientific studies conducted against this particular species of pest is very limited.This study is a preliminary report that shows the high insecticidal effect of *C.fistula* and *A.muricata* against *L.oratorius*. All aqueous extracts under study show a dose-response relationship between different concentrations of plants used in this study and their percentage mortality against the pest.

6. Conclusion

All the above results strongly suggests that the above mentioned plants are useful candidate for the development of biopesticides against the major rice pest *L.oratorius*. Secondary metabolites present in these plants have high bioactivity against this pest. Out of the five candidates studied, *C.fistula* and *A. muricata* and *A.heterophyllus* merit further evaluation.

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