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Insecticidal and repellent activities of *Clerodendrum serratum* L. leaf extract against rice weevil, *Sitophilus oryzae* L.

S. R. Yankanchi[1]*, A. D. Jadhav[1] and P. M. Patil[2]

1. Department of Zoology, Shivaji University, Kolhapur-416 004, India
2. Department of Agrochemicals and Pest Management, Shivaji University, Kolhapur-416004

E-mail : sryankanchi@yahoo.co.in Contact No : +91 - 9579692348

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Abstract

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), is one of the major stored-grain pests of cereals throughout world. During the present study an attempt was made to evaluate the insecticidal and repellent activities of crude methanol extract of *Clerodendrum serratum* L. against the rice weevil, *Sitophilus oryzae L*. Insecticidal activity was evaluated using a no-choice test with treated filter paper with wheat grains while repellency evaluated using a choice test with treated filter paper. The crude methanol extract was found to possess moderate insecticidal efficacy against *S. oryzae* with maximum mortality of 63% at 32 mg/mL (w/v) 7 days after exposure however toxicity depended on both concentration and duration of exposure. The methanol extract was highly repellent against *S. oryzae* ranging from 23 and 77% at 30 minutes to 2 h after exposure and from 47 to 97% at 4 to 24 h after exposure. The exposure period appeared to be the most important factor affecting the repellency activity of crude extract of *C. serratum.* Therefore, these leaves extracts may be suggested as admixtures in the integrated management of *S. oryzae* infestation of cereals during storage.

Key words : Biopesticides, *Clerodendrum serratum,* Insecticides, *Sitophilus oryzae*

INTRODUCTION

Insects have been causing remarkable losses not only to the crops growing in fields but also to post-harvest commodities during storage. Significant losses in grains, both quantitative and qualitative occur during storage and the damage caused by insects may amount to 5-10% in the temperate and 20-30% in the tropical zone [1]. The worldwide losses in stored grains due to insects and rodents have been estimated by food and agriculture organization (FAO) to be about 20%, the figures ranging from 10% in Europe and North America to 30% in Africa and Asia. It is estimated that more than 20,000 species of field and storage pests destroy approximately one-third of the world's food production, valued annually at more than $100 billion among which the highest losses (43%) occurring in the developing countries [2-3].

The rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae), is a major stored grain pest of economically important cereals such as rice, wheat, jawar and maize [4-5]. The female lays eggs into grains and larvae remain in the grain for their entire duration. Newly emerged adults may spend several days within the grain, before chewing exit holes to emerge [6-7]. The grains, therefore, become unsuitable for human consumption, to cause weight loss, decreased germination potential and reduction in commercial value of the seeds [8]. Serious problems of genetic resistance by insect species, pest resurgence, residual toxicity, phytotoxicity, vertebrate toxicity, widespread environmental hazards and increasing costs of application of the presently used synthetic pesticides have directed the need for effective, biodegradable pesticides. This awareness has created worldwide interest in the development of alternative strategies, and the use of plant products has become an alternative in protecting nature from pesticidal pollution. The efforts have been demonstrated by all and the various botanicals have been found effective against different stored grain pests [9-16]. Botanical insecticides are broad spectrum in pest control and many are safe to apply, unique in action, and can be easily processed and used. Plants contain a large number of secondary metabolites and those categorized under terpenoids, alkaloids, glycosides, phenols, tannins etc. play a major role in plant defense and cause behavioural and physiological effects on insects. Thus, in recent years an impetus has been on developing and evaluating botanical insecticides in view of their relative safety to the environment.

The *Clerodendrum serratum* L. belongs to family Verbenaceae, growing throughout India up to 1500 meters height including Eastern Himalaya, Nepal, Kumao, Bengal, Bihar and Western Ghats area in abundance. The bark and leaves contains three major triterpenoid constituent's oleonolic acid, queretaroic acid and serratagenic acid. The powdered stem contains D-mannitol, D-glucoside of sitosterol and sitosterol. The compounds identified are predominantly phenolics, particularly hydrolysable tannins and flavonoids. Glucose and D-mannitol, Oleanolic acid, Queretaroic acid and serratagenic acid are present in root bark of this plant whereas, stimasterol, α-spinasterol, luteolin, luteolin-7-0 glucuronide, apigenin, baicalin and scutellarin are found in leaf [17-18]. Biological activities revealed by saponin isolated from root bark of *C. serratum* caused release of histamine from lung tissue and also the alcoholic extract of roots showed a significant anti-inflammatory activity in carrageenan and also in the cotton pellet model in experimental mice, rats and rabbits [19-20]. Therefore, the present study was conducted to evaluate the insecticidal and repellent activities of crude methanol extract of *C. serratum* leaves against *S. oryzae* under laboratory conditions.

MATERIALS AND METHODS

Test insects:

The rice weevil, *Sitophilus oryzae* adults were collected from naturally infested wheat grains from a local market in Kolhapur, Maharashtra, India. The insects were reared on clean and un-infested wheat grains (*Triticum aestivum* L. var. NIAW-917). Two hundred adult insects were released in 1 kg wheat seeds in a

plastic jar capped with muslin cloth to ensure ventilation. After 48 h, the adults were removed and the jar was left for 45 days to obtain adult beetles and subsequently these beetles were used for the experiments. The jars were kept under maintained temperature (25±2°C), relative humidity (65-70%) and (12L: 12D) photoperiod. All experiments were conducted at these environmental conditions.

Preparation of crude extracts:

The healthy, mature and succulent leaves of *Clerodendrum serratum* L. (Verbenaceae) were collected in and around Kolhapur, India. Plant was identified using standard volume of Flora of Kolhapur district [21] and confirmed with the help of experts and the herbarium specimen (MMS 337) was deposited at the Department of Botany, Shivaji University, Kolhapur. The leaves were washed with distilled water and air dried for a week under shade and macerated using domestic grinder. Dried powder (2 kg) was subjected to methanol extraction with Soxhlet apparatus for 24 hrs and filtered with a Whatman (No.1) filter paper. The extract was concentrated by using a rotary evaporator under reduced pressure to give the crude methanol dark-green extract (98 g) which was used for bioassays.

Insecticidal test:

The insecticidal and repellent activities of *C. serratum* leaf methanol extract against *S. oryzae* was carried out with some modifications [3] with two bioassay methods (a) treated filter paper with wheat grains, (b) treated filter paper without wheat grains. Whatman (No. 1) filter paper discs (9 cm diameter) and 10 wheat grains which were placed at the center of each filter paper were treated with 1 mL of extract at the concentrations of 2, 4, 8, 16 and 32 mg/mL made in analytical grade of acetone whereas, controls received the same volume of carrier. The solvent was allowed to evaporate completely at room temperature. Each filter paper disc with wheat grains was then placed on a glass Petri dish (9 cm diameter) and 10 unsexed *S. oryzae* adults (0-24 days old) were introduced into the center of each Petri dish before wrapping with plastic wrap and covered up with its lid (10 petri dishes/replication, 3 replications/ concentration). The number of dead insects was recorded after 1, 2, 3, 4 and 7 days treatment.

Repellency test:

The repellency test of *C. serratum* extract was determined in glass Petri dishes (9 cm in diameter). Whatman (No.1) filter paper of 9 cm diameter was divided into two equal parts. Test solutions were prepared by diluting the crude extract in acetone to various concentrations (2, 4, 8, 16 and 32 mg/ml and applying 500 µl of each solution which produced the equivalent to 0.063, 0.126, 0.252, 0.503 and 1.007 mg/cm2). The control half was treated with the same volume of carrier. Both treated and control parts were allowed to dry completely at room temperature. Then, the treated and control parts were connected with clear adhesive tape and placed alternately on each Petri dish. Ten wheat grains were then placed on both treated and control parts and ten unsexed *S. oryzae* adults were released at the center of the filter paper disc. Plastic wrap was used to wrap each Petri dish before its cover was placed (10 Petri dishes/ replication, 3 replications /concentration). The number of insects presented in the control (N control) and treated (N treated) sides of the disc were counted at 30 min, 1h, 2h, 4h, 8h, 16h and 24h after exposure.

Statistical analyses:

For toxicity test, *S. oryzae* mortality was expressed as a percentage, whereas repellency test was evaluated using the following formula: Percentage Repellency (PR) = NC/NC+NT × 100, where NC was the number of insects on the control side and NT was the number of insects on the treated side. All observations were corrected by using the Abbott's formula [22] before analysis. The data for percentage of repellency were square root arcs in transformation before running the ANOVA using SPSS program, and treatment means were compared by the Least Significant Difference (LSD) at $P = 0.05$.

RESULTS

The crude methanol leaf extract of *Clerodendrum serratum* demonstrated moderate insecticidal activity against *S. oryzae* adults with wheat grains (Table -1). When the insects were released on filter paper without wheat grains there was no significant mortality at any of the concentrations after 7 days, due to that results are not mentioned. In general, percentage of mortality was increased with increasing the concentration of plant extract and exposure time. The mortality of the *S. oryzae* is perhaps caused by feeding on the wheat grains treated with extract. There were no significant differences in mortality between the controls and 2, 4, 8, 16 and 32 mg/mL. At the highest concentration (32 mg/mL) of crude methanol extract revealed significantly higher mortality of 63.3% than other doses.

The *Clerodendrum serratum* extract was highly repellent to *S. oryzae* (Table 2). There were significant difference between times, however no significant effect of concentrations and/or interaction between concentration and time. The crude methanol extract at all concentrations demonstrated repellency effect against *S. oryzae* ranging from 23-67% 30 minutes after releasing insects. At 1 to 2 h after release, the extract showed repellency ranging from 33-73%. As the time progressed, the extract showed fluctuation in repellency between 60-97% at 4 to 24 h after release. At the end of experiment (24 h), the concentration of 0.503 mg/cm^2 showed maximum repellency activity (97%) which was significantly different from that after 1h, 2h, 4h, 8h, and 16 hours. The other concentrations showed repellency ranging from 70-90% as compared to control set.

DISCUSSION

The toxicity of *Clerodendrum serratum* extract increased with both concentration and exposure time. This phenomenon has also been reported for most of the botanical insecticides against *S. oryzae* [7, 23- 24] wherein, they were evaluated ethanol extracts (2.5 and 5%) of different *Clerodendrum* species leaves against *S. oryzae.* The results recorded the effectiveness up to 21 days which were in confirmation with the present findings. The toxicity of *C. serratum* extract increased with both concentration and time. The repellent efficacy of the crude methanol extract of *C. serratum* could be attributed to the mixture of compounds detected by the *S. oryzae* adults. The obtained results can be explained by the fact that the constituents of the crude methanol extract of *Clerodendrum serratum* are high molecular weight compounds with low volatility. It is evident from this experiment that time is the main factor for repellency of *S. oryzae* by the *C. serratum* extract. Similar results were also found for the hexane leaf extract of *Solanum argentinum* Bitter et Lillo (Solanaceae) where 10 mg/mL or 0.31 mg/cm2 showed repellency of 15, 60, 70, 80 and 90% at 1, 2, 3, 4 and 5 h after exposure, respectively on *S. oryzae* adults by using the filter paper test [25].

Interestingly, *C. serratum* extract showed relatively lower insecticidal activity than *C. inerme*, even though both plants belong to the same family Verbenaceae [26, 7]. The toxicity of

Table 1 : Insecticidal (%) activity of methanol leaf extract of *Clerodendrum serratum* against *Sitophilus oryzae* adults using treated filter paper and wheat grains

Concentration (mg/mL)	Days after treatment				
	1	2	3	4	7
(Control)	0.0 ± 0.0a	0.0 ± 0.0b	3.3 ± 2.3b	3.3 ± 2.3b	3.3 ± 2.3b
2	0.0 ± 0.0a	0.0 ± 0.0b	3.3 ± 2.3b	10.7 ± 2.6b	10.7 = 2.6b
4	0.0 ± 0.0a	0.0 ± 0.0b	6.7 ± 2.7ab	16.7 ± 3.3ab	16.7 ± 3.3ab
8	0.0 ± 0.0a	3.3 ± 0.9b	10.0 ± 2.7ab	23.3 ± 3.5ab	20.0 ± 3.6ab
16	0.0 ± 0.0a	6.7 ± 3.3a	23.3 ± 3.7a	30.0 ± 4.0a	36.7 = 6.7a
32	3.3 ± 3.3a	6.7 ± 1.9a	30.3 ± 3.7a	57.3 ± 4.3a	63.3 = 4.7a

Means ± SE within the column followed by the same letters are not significantly different by LSD ($P = 0.05$).

Table 2 : Repellency (%) activity of the methanol leaf extract of *Clerodendrum serratum* to *Sitophilus oryzae* adults using treated filter paper

Concentration of extract (mg/cm^2)	Duration of exposure						
	30min	1h	2h	4h	8h	16h	24h
Control	0.0±0.0c	0.0±0.0d	0.0 ±0.0c	0.0±0.0c	0.0 =0.0c	0.0±0.0c	0.0 =0.0c
0.031	23.3±3.2bc	33.3 ± 3.7c	33.3±3.7b	47.0±7.1bc	53.3±12.2b	60.0±12.4ab	77.0±7.8b
0.063	43.3±5.6b	47.0± 6.3c	57.0±9.2b	60.0±12.2b	60.0±12.2b	67.0=7.6ab	73.3±7.8b
0.126	47.0±11.3b	60.3±7.0ab	63.0±6.6b	67.0±9.0b	67.0±7.6b	73.0=9.2ab	80.0±9.3ab
0.252	50.0±12.6b	63.0±6.5a	67.0±13.3a	67.3±8.6b	73.3±13.3ab	77.0=6.3b	90.0±12.0a
0.503	53.3 ±9.6b	73.3±9.0a	77.0±7.6a	80.0±16.2a	80.3±13.3ab	87.0±9.8a	97.0±13.3a
1.007	67.0±13.0a	73.3±5.9a	73.0±10.3a	77.3±12.0a	90.0=16.0a	83.3±12.0a	87.0±8.0a

Means ± SE within the column followed by the same letters are not significantly different by LSD ($P = 0.05$).

Clerodendrum serratum extract could be caused by the bioactive compounds present in the extract. The chemical examination of methanol extract of *C. serratum* leaves mixture contains three major triterpenoid constituents oleonolic acid, queretaroic acid and serratagenic acid. The root bark yields a glycoside material, phenolic in natures. D-Mannitol is isolated from the bark with a yield of 10.9 %. The powdered stem contains D- mannitol, D-glucoside of sitosterol, sitosterol and acetyl alcohol [20]. Some of these compounds and their derivatives have been previously reported for their insecticidal properties against stored-product pests. Roychoudhary [23] reported that oleanolic acid, a major component of *Junellia aspera* (Verbenaceae), exhibited toxic effects by ingestion on *S. oryzae* and direct application on the cuticle of *T. castaneum* adults. On the other hand, benzaldehyde, which occurs in peach and almond kernels, was found to have potent fumigant toxicity against *S. oryzae* whereas 4-ethoxybenzaldehyde is less toxic towards the same weevils [27]. Flavonoids are toxic to adult *Callosobruchus chinensis* (L.) via contact on the filter paper bioassay [28].

CONCLUSION

This study showed that toxicity and repellent activities of the *C. serratum* leaf methanol extract against *S. oryzae* adults depended on several factors including chemical constituents of the extract, insect species and exposure time. This extract may act as a repellency rather than oral toxicity against *S. oryzae* which may be a better way for managing this insect. The impact that larvae of this weevil stay inside the grain for a long time before emerge to adult, it is difficult to control them with the insecticide while the chemical repellent can protect the grains and reduce the initial infestation, mentioned by Highland and Cline [29]. Moreover, low toxicity of this crude extract also leads to less subsequent resistance of pest, however, the fact that this mixture has low toxicity needs more work. In addition, more research is required to determine stability, duration of effectiveness, effect on end use quality, toxicity to human, contact toxicity with treated grain and effect of extract on different species of stored-product insects.

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