



PHYTOCHEMICAL SCREENING AND EVALUATION OF THE BIOCONTROL ACTIVITY OF SELECTED LEAF EXTRACTS AGAINST THE RICE WEEVIL, *SITOPHILUS ORYZAE*

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ABSTRACT

Plants are considered to be the richest source of highly renewable natural insecticides. Specifically, botanical extracts provide an interesting alternative to synthetic pesticides and are safe and compatible. In the current study, isopropyl alcohol extracts of *Nerium oleander* and *Datura stramonium* leaves were tested for its efficacy in inducing mortality of rice weevil *Sitophilus oryzae*. The mortality percentage was tested at concentrations of 2%, 4%, 6%, 8% and 10%. The results were subjected to statistical analysis (Mean \pm SD). Further, phytochemical analysis of the leaf extracts were carried out using standard protocols. Results of the biocontrol activity revealed that mortality rate increased with increasing concentration. Among the two leaf extracts, *Datura stramonium* leaf extracts exhibited maximum biocontrol activity. *Datura stramonium* leaf extracts when tested for the phytochemical revealed the presence of Alkaloids, Tannins, Steroids, Saponnins and Cardiac glycosides. It may be concluded on the basis of the result of present study that isopropyl alcohol extracts of plants have toxic effects with significant insecticidal effects.

Keywords: Biocontrol activity, Plant extracts, Solvents, Rice weevil, *Sitophilus oryzae*, Phytochemical analysis.

1. INTRODUCTION

Pest management is facing economic and ecological challenge worldwide due to human and environmental hazards caused by majority of the synthetic pesticide chemicals. Botanical insecticides have long been used as an attractive alternative to synthetic chemical insecticides for pest management, because synthetic insecticides cause threat to the environment and to human health. Homemade botanical insecticides are mostly being used by people and farmers in low-income countries. Their use is often due to the limited availability and high rate of commercial pesticides. Homemade botanical insecticides are recommended by agricultural programmes and some development organizations [1].

Damage caused by pests and arthropods to crops in particular, can lead to even higher losses in yield or total crop failure [2-4]. Plant extracts kill and can repel pests and affect insect growth and development, and have anti-feedant and growth arresting effects. Continuous and indiscriminate use of pesticides has not only led to the development of resistant strains but also accumulation of toxic residues on food grains used for human consumption [5]. Plant derived materials are less likely to contaminate the environment, more readily biodegradable, and may be less toxic to animals and human

beings. Therefore, today, researchers are seeking new classes of naturally occurring insecticides that might be compatible with newer pest control approaches [6-8].

Plant extracts as bio-control agents in pest management, have been reported to be eco-friendly, safer, less costly and more compatible with environmental components, compared to synthetic insecticides and are now called as "green agrochemicals" and its use is also increasing now a days. [9]. Therefore, botanical extracts as biological control agents has more advantages than synthetic insecticides. The present study investigated the bio-control activity of *Nerium oleander* and *Datura stramonium* against the pest *Sitophilus oryzae*.

2. MATERIAL AND METHODS

2.1. Studies on the potency of selected botanicals

2.1.1. Collections of test materials

Leaves of *Nerium oleander* and *Datura stramonium* were collected from Kallekulangara, Palakkad District, Kerala, and the specimens were identified, certified with the voucher specimen number, *Nerium oleander* (KFRI/Bot/Heb-2021/3-6) and *Datura stramonium* (KFRI/Bot/Heb-2021/3-1) from the Kerala Forest Research Institute Peechi, India, Thrissur, Kerala.

2.1.2. Preparation of leaf powder and extracts

Fresh leaves of *Nerium oleander* and *Datura stramonium* were collected, and air dried under shade and powdered using an electric pulverizer. These powders were subjected to extraction [10, 11] using the solvent isopropyl alcohol. The leaves extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40°C.

The residue thus obtained was stored in the refrigerator for further use. Biocontrol activity and phytochemical analysis activity of the leaves of *Nerium oleander* and *Datura stramonium* were investigated.

2.1.3. Test insect

The test insect was *Sitophilus oryzae* (Fig. 1). It was collected from infected stored rice in home. The adult insects were separated from the mixed population through hand picking and sieving method. Then the collected adult was kept in a box with ventilation. The box was provided with rice as a feed for the insect. The collected insects are carefully stored from high temperature and humidity.



Fig. 1: Test insect - *Sitophilus oryzae*

2.2. Biocontrol activity of plant extracts

A pilot study was carried out to assess the biocontrol efficacy of leaf extracts of selected plant against the insect *Sitophilus oryzae*. Effective doses were determined first and then detailed investigation was carried out. The experimental set up consisted of five concentrations each with three replications. Simultaneously control was also maintained. Petridishes were taken and marked with different concentrations. Concentrations of 2%, 4%, 6%, 8% and 10% were applied in filter paper and placed inside the petridishes for determining the biocontrol efficacy of the *Nerium oleander* and *Datura stramonium* leaf

extracts. For the control treatment, the filter paper was spread with distilled water. In each of the petridish, ten *Sitophilus oryzae* insects were placed. The observation period was one day (24 hours).

2.2.1. Percentage mortality

To observe the mortality, different concentrations of leaf extracts that was applied on the filter paper was allowed to dry. Then it was placed in a petridish and ten *Sitophilus oryzae* insects were placed inside each petridish including the control. Petridish was closed. It was observed after 3h, 6h, 9h, 12h and 24 hours. The number of dead insects were counted and recorded.

Percentage mortality = $\frac{\text{Number of insects dead}}{\text{Total number of insects}} \times 100$

2.2.2. Statistical analysis

Standard deviation was calculated for the data which was obtained from the test. Each value ($\bar{x} \pm SD$) represents average of three replications.

2.3. Phytochemical analysis of leaf extracts

The plant extract was assessed for the existence of alkaloids, tannins, phenols, flavonoids, sterols, terpenoids, saponins, reducing sugars, phytosterols and cardiac glycosides by the phytochemical analysis using typical standard methods [12-18].

3. RESULTS AND DISCUSSION

3.1. Biocontrol activity

Because of the residue problem and health risks to consumers, the use of synthetic chemical insecticides is either not permitted or used restrictively. Plants may provide a potential alternative to the currently used synthetic insecticides, they constitute a rich source of bioactive molecules [19].

3.1.1. Biocontrol activity of *Nerium oleander* leaf extract

In the present study biocontrol activity of leaf extracts of *Nerium oleander* against test insect *Sitophilus oryzae* was carried out. Results of biocontrol activity of leaf extracts of *Nerium oleander* was shown in fig 2.

The result showed that *Nerium oleander* showed high biocontrol activity at 10% and 8% concentration against *Sitophilus oryzae*, by providing 100% insect mortality. The mortality percentage was 36%, 41%, 66%, 100% and 100% percentage at concentration 2, 4, 6, 8 and 10% concentrations respectively. Lowest biocontrol activity was seen at the lowest concentration of 2%

concentration that provided only 36% total insect mortality.

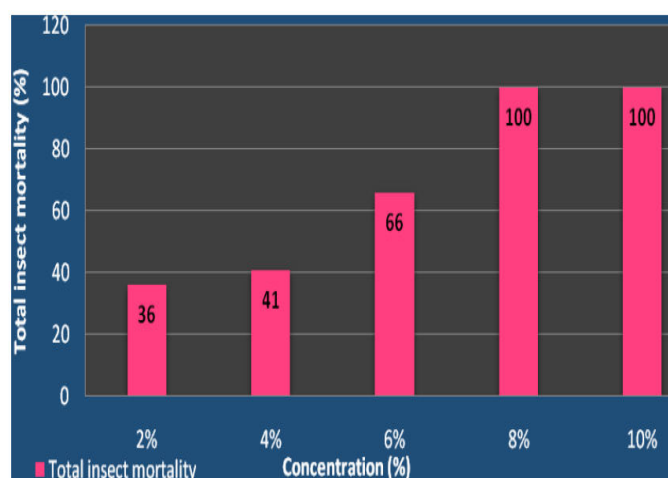


Fig. 2: Graph Showing the Biocontrol Activity of *Nerium oleander* Leaf Extract against the Test Insect *Sitophilus oryzae*

It was noted in the present study that as concentration increased, the biocontrol activity of *Nerium oleander* leaf extracts was also found to increase. It was also seen that as study time increased, the mortality rate of test insect *Sitophilus oryzae* increased.

In the present study, isopropyl alcohol extract of *Nerium oleander* leaf, tested at different concentrations (2, 4, 6, 8, 18 and 10 %) and at different hours (3h, 6h, 9h, 12h and 24h) showed biocontrol activity against *Sitophilus oryzae* giving a mortality percentage of 36%, 41%, 66%, 100% and 100% respectively. Similar study was carried out by Rajasekhar Reddy and Usha Rani [20] that reported that *C. maxima* crude extract was found to have provided high effectiveness against the stored product insects, *S. oryzae* and *R. dominica* causing 100% mortality. Previously, *C. maxima* seeds were shown to have diversified biological functions, such as insecticidal properties against certain agricultural pests [21]. Repellent, ovicidal and larvicidal, activities of the leaf extract of *C. maxima* plants against the mosquito *Culex quinquefasciatus* were recorded by Mullai and Jebanesan [22].

3.1.2. Biocontrol activity of *Datura stramonium* leaf extract

In the present study biocontrol activity of leaf extracts of *Datura stramonium* against test insect *Sitophilus oryzae* was carried out. The results of biocontrol activity of leaf extract of *Datura stramonium* was shown in Fig 3.

The result of the present study showed that *Datura stramonium* leaf extract exhibited maximum biocontrol activity at 10 %, 8% and 6% concentration, thereby providing 100% insect mortality. At concentration of 2%, 4%, 6%, 8% and 10% the mortality percentage showed by *Datura stramonium* leaf extract against test insect *Sitophilus oryzae* was 51%, 72.65%, 100%, 100% and 100% respectively. The lowest biocontrol activity was seen at the lowest concentration of 2 % that provided only 51% total insect mortality.

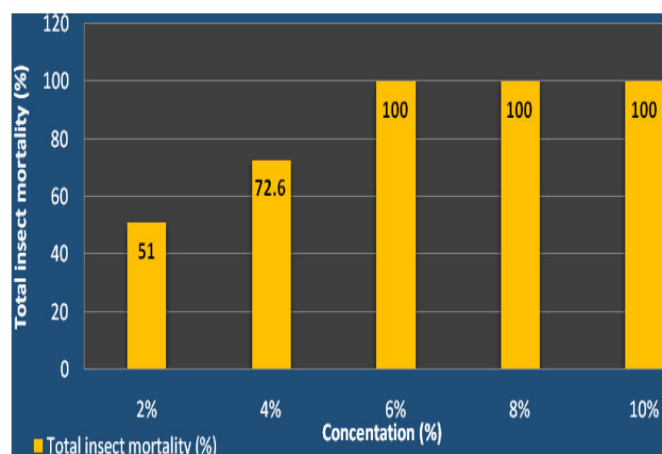


Fig. 3: Graph Showing the Biocontrol Activity of *Datura stramonium* Leaf Extract against the Test Insect *Sitophilus oryzae*

In the present study, isopropyl extract of *Datura stramonium* leaf showed excellent biocontrol activity against the test insect *Sitophilus oryzae*. Observations parallel to the present study was recorded by Tripathi *et al.*, [23] in that it was observed that essential oils of *Artemisia* species showed toxic, repellent and development inhibitory activities against two economically harmful stored insects. The results are in tune with the findings of Tunc *et al* [24], who reported that the essential oil vapours distilled from *Eucalyptus* were reported as fumigants and caused 100% mortality of the eggs of stored product pests.

Biological control against the cowpea weevil using essential oils of some medicinal plants was carried out by Righi *et al.*, [25]. The amount of 20 μ l of *Artemisia* oil had a lethal effect on the males and the females. There was 100% mortality noted with the use of *Artemisia* oil after just a few minutes of treatment. In accordance with these results the results of the present study also revealed that the leaf extracts of *Datura stramonium* exhibited 37% mortality within 3 hours of treatment. Ketoh *et al* [26]

showed that monoterpene β -pinene and the piperitone in *A. herba* have insecticidal activity against *C. maculatus*.

The result showed that *Nerium oleander* showed high biocontrol activity within 9 hours in 10 % concentration against *Sitophilus oryzae*, by providing 100% insect mortality. De-Souze and Vendramim [27] and Mazen et al., [28] has reported that *N. oleander* (5%) has adversely affected the survival of immature stages of *P. fuscipes*. The 5% ethanol leaf extract of *N. oleander* when subjected to higher deleterious effects caused heavy mortality presumably either on accounts of its insecticidal, larvicidal, antifeedant properties and repellent effects as reported in case of *Bemisia tabaci*. Few researchers have reported that *N. oleander* has effectiveness in providing high mortality in *Rhyzopertha dominica* [29].

In the present study to test the biocontrol activity of *Nerium oleander* and *Datura stramonium*, highest biocontrol activity was showed by *Datura stramonium* leaf extract. Maximum biocontrol activity was exhibited by *Datura stramonium* at 10 %, 8 % and 6 % concentration, thereby provided 100% insect mortality. It may be due to the presence of chemical constituents in the leaf extract. In a similar study Dunkel et al., [30] observed more than 70% mortality for adult *Sitophilus oryzae* was noted when it was exposed for 2 weeks to wheat treated with the neem-based insecticide.

3.2. Phytochemical analysis

Plants are considered as rich sources of bioactive chemicals and there may be an alternative source of insect control agents [31]. Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and have different activities observed by many researchers [32, 33].

3.2.1. Phytochemical analysis of *Nerium oleander* leaf extracts

In the present study phytochemical analysis of isopropyl alcohol extract of *Nerium oleander* leaves that exhibited moderate biocontrol activity against the insect *Sitophilus oryzae* were carried out. Results of phytochemical analysis of *Nerium oleander* was shown in the table 1. The results of phytochemical analysis of *Nerium oleander* showed the presence of Alkaloids, Reducing sugars and Saponnins. Whereas Flavonoids, Aminoacids, Tannins, Steroids, Phenols, Phytosterols and Cardiac glycosides were found to be absent.

3.2.2. Phytochemical analysis of *Datura stramonium* leaf extracts

In the present study, phytochemical analysis of isopropyl alcohol extract of *Datura stramonium* leaves that exhibited maximum biocontrol activity against the insect *Sitophilus oryzae* were carried out. Results of phytochemical analysis of *Datura stramonium* was shown in the table 1. The results of phytochemical analysis of *Datura stramonium* showed the presence of alkaloids, tannins, steroids, saponnins and cardiac glycosides. Whereas flavonoids, aminoacids, reducing sugars, phenols and phytosterols were found to be absent.

Table 1: Phytochemical Compounds Present in *Nerium oleander* and *Datura stramonium* Leaf Extract

Sl. No.	Constituents	Isopropyl Alcohol Extract	
		<i>Nerium oleander</i>	<i>Datura stramonium</i>
1	Alkaloids	+	+
2	Flavonoids	-	-
3	Aminoacids	-	-
4	Tannins	-	+
5	Reducing sugars	+	-
6	Steroids	-	+
7	Phenols	-	-
8	Saponnins	+	+
9	Phytosterols	-	-
10	Cardiac glycosides	-	+

“+” Presence, “-” Absence

In a similar study carried out by Farina et al., [34] it was found that phytochemicals in the leaves of aqueous and methanol extracts of *Aegle marmelos* revealed the presence of many phytochemicals such as tannins, alkaloids, flavonoids, terpenoids, saponins, cardiac glycosides, carotenoids and reducing sugars. Phytochemicals present in the ethanol, petroleum ether, chloroform, and methanol extract of a single unidentified variety of *Aegle marmelos* have been previously reported by Venkatesan et al., [35] Kothari et al., [36].

Results in concordance with the present study were reported by Durai et al., [37] that observed that qualitative analyses of bioactive compounds for the three crude extracts of *S. macrophyllathere* showed a wide range of phytochemical compounds present in the extracts. The data revealed that the strong positive results were found for alkaloids, terpenoids and carbohydrate in both

methanol seed and leaf extracts. The strong positive results for tannins were found in leaf and central-fruit axis. Steroids and Amino acids were positively found in all three extracts.

Studies in relation to phytochemical analysis were carried out by many researchers. Phytochemical screening of ethanol extracts of *O. gratissimum*, *S. acuta*, *T. occidentalis* and *V. amygdalina* were carried out by Adeniyi et al., [38]. Results showed the presence of alkaloids, tannins and flavonoids in the extracts. Phlobatannins and terpenoids were detected in the ethanol extract of *O. gratissimum*, *S. acuta* and *V. amygdalina* while cardiac glycosides were detected in the ethanol extract of *O. gratissimum*, *S. acuta* and *V. amygdalina*. Also, saponins were detected in both *T. occidentalis* and *V. amygdalina* whereas steroids were seen only in the ethanol extract of *S. acuta* and *T. occidentalis*. Kabarau and Gichia [39] have recorded that the phytochemicals present in the plant extracts may be responsible for their insecticidal properties of them.

Similarly in the present study isopropyl alcohol extract of *Datura stramonium* exhibited high biocontrol activity than *Nerium oleander* leaf extract and showed the presence of Alkaloids, Tannins, Steroids, Saponins and Cardiac glycosides. The phytochemicals present in the selected leaf extracts might be responsible for its biocontrol activity. The current findings are also parallel to the report of Qaisar et al., [40] that has recorded that *E. prostrata*, when subjected to phyto-chemical screening revealed the presence of flavonoids, terpenoids, and tannins.

4. CONCLUSION

India is basically an agro-based country; more than 80% of Indian population depends on agriculture. Indian economy is largely determined by agricultural productivity. Insect pests cause significant damage to crops and affect agricultural productivity. Plants have been used to control agricultural pests as an alternative to synthetic chemicals which are harmful to man and environment.

The plants including *Nerium oleander* and *Datura stramonium* leaf extract have a high potency to control the pest due to the presence of phytochemical and bioactive components. On the basis of above findings, percentage mortality may due to the presence of bioactive compounds present in the plant extract so it is effective against the pest. The selected plants such as *Nerium oleander* and *Datura stramonium* had a toxic effect against the stored grain pest *Sitophilus oryzae*.

On the basis of the result of present study suggested that isopropyl alcohol extracts of plants have toxic effects with significant insecticidal effect and could be a potential tool to protect stored grains against *Sitophilus oryzae*, and this may contribute to a reduction in the application of synthetic insecticides, which in turn increases the opportunity for natural control of various pests by using plant pesticides.

Conflict of interest

There is no conflict of interest among the authors

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