



Guardians of the grains: evaluating the biopesticidal potential of certain plants extracts against the Rice Weevil, *Sitophilus oryzae* L.

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Abstract

Rice (*Oryza sativa* Linn.) is a vital cereal crop and a staple food for a significant portion of the global population. However, the rice weevil (*Sitophilus oryzae*) poses a serious threat to rice storage, causing substantial losses and impacting food availability. The current methods of controlling rice weevils primarily rely on the use of chemical insecticides, which have led to concerns about resistance development and adverse effects on human health and the environment. Therefore, there is a growing interest in exploring alternative approaches using plant-natural products as less harmful pest control agents. In this study, we assessed the repellent and toxicity efficacy of crude methanolic leaf extracts from three plant species, namely *Caesalpinia pulcherrima*, *Clitoria ternatea*, and *Bryophyllum pinnatum*, against adult rice weevils (*S. oryzae*). The phytochemical analysis revealed the presence of alkaloids, flavonoids, polyphenols, and terpenoids in high quantities in both extracts. The repellency bioassay demonstrated that all three plant extracts exhibited effective repellent activity against rice weevils in a time-dependent manner. *Br. pinnatum* showed the highest repellency after 120 minutes of exposure, followed by *Cl. ternatea* and *C. pulcherrima*. Furthermore, the contact toxicity bioassay demonstrated concentration-dependent toxicity of the extracts, with *Br. pinnatum* exhibiting the highest toxicity. Probit regression analysis provided the LC₅₀ (lethal concentration) and LC₉₀ values for each extract, indicating their efficacy. Overall, the results suggest that these plant extracts have the potential to serve as natural pesticides for managing rice weevils, offering an alternative to synthetic insecticides.

Keywords: Rice weevil, *Sitophilus oryzae*, *Caesalpinia pulcherrima*, *Clitoria ternatea*, *Bryophyllum pinnatum*, repellency, contact toxicity, natural pesticides

Introduction

Rice, *Oryza sativa* (Linn.), is one of the economically important cereals and serves as the most important staple food for half of the world's population, cultivated in over 100 countries (Okon, *et al.*, 2012^[1]; Ranganathan, *et al.*, 2018)^[2]. However, insect pests cause significant losses in rice storage, affecting food availability for many people. The rice weevil, *Sitophilus oryzae*, is a highly destructive pest that poses a significant threat to stored cereals worldwide, especially in tropical regions, infesting stored grains such as rice, wheat, maize, and split peas globally (Follett, *et al.*, 2013^[3]; Schuster and Torero, 2016^[4]; Park *et al.*, 2003^[5]; Yoon *et al.*, 2007^[6]). The rice weevil, *S. oryzae*, causes damage by creating bores in kernels, and hollowing out the infested grain (Srivastava and Sabtharishi, 2016)^[7]. This pest feeds on carbohydrates in rice grains, leading to weight loss, contamination, and reduced germination (Park, *et al.*, 2003^[25]; Okram and Hath, 2019^[8]). Moreover, increased temperature and humidity in infested grains create favorable conditions for secondary pests and pathogens (Srivastava and Sabtharishi, 2016)^[7]. Infestations by this insect lead to substantial losses in cereal crops, resulting in compromised grain quantity, quality, and seed viability (Gupta *et al.*, 1999^[9]). In India alone, post-harvest losses of food grains are estimated to be between 12 to 16 million MT per year, leading to an annual loss of Rs 92,651 crore (Singh, 2010^[10]; Akhtar, *et al.*, 2017^[11]; Pandey, 2018)^[12].

Currently, the primary means of controlling stored-product pests, including *S. oryzae*, relies on the application of

insecticides such as organophosphorus compounds, pyrethroids, and fumigants like phosphine (PH₃) (Kljajic and Peric, 2006^[13]; Ren *et al.*, 2008)^[14]. Fumigation remains one of the most effective methods for managing stored-product insects. While chemical fumigants and synthetic insecticides have been commonly used to prevent grain losses, their repeated use has resulted in pest resistance and raised concerns about adverse effects on food quantity, quality, human health, and the environment (Kim, *et al.*, 2015^[15]; Liu, *et al.*, 2016^[16]; Ozkara, *et al.*, 2016^[17]; Nayak *et al.*, 2007^[18]; Rajendran and Sriranjini, 2008)^[19]. Therefore, there is a need to develop insect-specific insecticides and gain a better understanding of the life cycle dynamics of *S. oryzae* (Merville, *et al.*, 2014)^[20].

Consequently, there is a growing interest in developing alternative approaches for safeguarding stored products, with a particular focus on utilizing plant natural products that are less harmful to both human health and the environment. Rahman, *et al.*, (2021)^[21] studied the pesticidal activity of methanolic extracts of five Sundarban mangrove plants as potential sources of natural pesticides against *S. oryzae* and *S. zeamais*, using a direct contact feeding deterrent wafer disc method. Hasan, *et al.*, (2016)^[22] evaluated the pesticidal effect of root extracts of *Coccinia grandis* against *S. oryzae* adults. Rani, *et al.* (2019)^[23], investigated, the toxicity efficacy of the sweet flag rhizome extract against *S. oryzae*. Jawalkar and Zambare (2020)^[24] evaluated the insecticidal properties of dry leaves of *Vitex negundo* against the stored grain insect pest *S. granarius*.

The present study aims to assess the repellent and toxicity efficacy of crude methanolic leaf extracts of *Caesalpinia pulcherrima*, *Clitoria ternatea*, and *Bryophyllum pinnatum* against the adult stages of rice weevil, *S. oryzae*.

Materials & Methods

Culturing of Insects: Rice weevils (*Sitophilus oryzae* L.) were collected from the old stock of rice grains from organic rice stores located in Zaheerabad, Telangana state, India. The insects were reared on sterilized whole wheat at a temperature of 26 ± 1 °C and relative humidity of $65 \pm 5\%$ in complete darkness. The first-generation adult insects were used in the repellent and contact toxicity bioassays.

Plant Materials

The leaves of three plant species (*Caesalpinia pulcherrima*, *Clitoria ternatea* & *Bryophyllum pinnatum*) were collected during their flowering stages from various locations in Zaheerabad, Telangana state, India. The leaves were thoroughly cleaned with distilled water and shade dried for 15 days. The fully dried leaves were ground into a fine powder using mortar first and later an electrical blender.

Extraction of Phytochemicals

To extract phytochemicals from the prepared powders, 100 gr. of each powder was added to 250 mL of methanol separately into a reagent bottle. The bottles were given frequent shakings for four days. On the fifth day, the solutions were filtered using Whatman filter paper no.1. The filtrates were kept under a fan to let the methanol dry. The semi-solid extracts were collected into plastic vials and stored in the refrigerator at 4°C until usage in phytochemical analysis and bioassays.

Phytochemicals Analysis

Various tests were conducted to identify the presence of secondary metabolites in the prepared extracts. The following tests were employed

Alkaloids: The presence of alkaloids was determined using the Mayor's Test. The test involved adding the Mayor's reagent to the extract and observing the formation of a cream-colored precipitate. The formation of this precipitate is indicative of the presence of alkaloids.

Flavonoids: Flavonoids were identified using the Alkaline Reagent Test. In this test, the extract was treated with sodium hydroxide (NaOH). The development of yellow color indicates the presence of flavonoids.

Terpenoids: The Salkowski Test was conducted to identify terpenoids. Concentrated sulfuric acid (H₂SO₄) was added to the extract, and the formation of a red or orange color indicated the presence of terpenoids.

Saponins: The Froth Test was employed to detect the presence of saponins. The extract was vigorously shaken, and the formation of foam indicated the presence of saponins.

Glycosides: The presence of glycosides was determined using the Keller-Killiani Test. The extract was treated with hydrochloric acid (HCl) and ferric chloride (FeCl₃). A red

or violet color development confirmed the presence of glycosides.

Polyphenols: The NaOH Test was conducted to identify the presence of polyphenols. NaOH was added to the extract, and the development of a yellow color indicated the presence of polyphenols.

Repellent bioassay

The repellency was tested according to McDonald *et al.* (1970) [25]. Half filter paper discs (Whatman n° 40, 9 cm diam.) were prepared and 20 mg of each extract was diluted in 1 ml of methanol at the concentration of 2%. A volume of 200 µl of each concentration was applied separately to one half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 200 µl of methanol. Both the treated and the control halves were allowed to dry out as exposed in the air for 10 min. Each treated half-disc was then attached lengthwise, edge-to-edge, to a control half disc with adhesive tape and placed in a Petri dish (9 cm diameter). Twenty adult insects were released in the middle of each filter-paper circle. Each extract was replicated five times. Insects that settled on each half of the filter paper disc were counted after 15 min, 30 min, 1h and 2h. The average of the counts was converted to percentage repellency (PR) using the formula of McDonald *et al.* (1970) [25].

$$PR = \left[\frac{Nc - Nt}{Nc + Nt} \right] \times 100$$

Where

Nc: number of insects in control test

Nt: number of insects in the treated test.

The mean repellency value of each extract was calculated and assigned to repellency classes from 0 to V:

class 0 (PR ≤ 0.1%), class I (PR = 0.1 - 20%), class II (PR = 20.1 - 40%), class III (40.1- 60%), class IV (60.1 - 80%) and class V (80.1 - 100%).

Contact Toxicity Bioassay

For conducting contact toxicity bioassays 1000 ppm of stock solutions were prepared for each extract by dissolving 1 gr. of extract in 10 mL methanol and 990 mL of distilled water. Later test solutions (100 ppm, 200 ppm, 300 ppm, and 400 ppm) were prepared by serial dilution method. Test solutions were prepared with same solvents excluding solutes. Cushnie and Lamb (2005) [26] methodology was followed to conduct Contact Toxicity bioassay. Filter paper sheets of 3 Cm diameter were impregnated with 1 mL of test solutions separately. After the test solutions are spread all over the filter paper, they were placed into separate petri dishes. Control sheets were impregnated by Control solution. 25 adult insects of *S. oryzae* were released into each Petri dish after the filter papers were air dried. Five replicates were maintained for each test sample. The plates were wrapped with plastic film and sealed with wax to prevent escape of insects. Small holes were made on the plastic film for aeration. Mortality counts were taken after 24hr, 48hr, and 72hr intervals of treatment and expressed as percentages of mortality. Dead insects were counted, death being ascertained by the complete absence of movement. Abbott's (1925) [27] formula was used to calculate the corrected mortalities.

Statistical Analysis: The mortality of each concentration was determined as the mean of four replicates after 24 hours. Probit analysis (Finney 1971) [28] was performed on the mortality and enzyme inhibition percentages to obtain the LC50 and I50 values, using MS Excel software. The LC50 and I50 values were considered significantly different if the 95% confidence limits did not overlap.

Results

The phytochemical analysis results of the two extracts (Table 1), revealed the presence of alkaloids, flavonoids, polyphenols, and terpenoids in high quantities in both extracts. Saponins were present in slight quantities in the two tested extracts. Glycosides were identified in *C. limon* extracts whereas they were absent in *A. vera* extracts.

The repellent bioassay results of the present study (Tables 2 and Figures 1) indicated that all the three plant extracts exhibited efficient repellent activity against the adult stages of *S. oryzae* in a exposure time-dependent manner. Highest repellency of all the three extracts was exhibited by *Br. Pinnatum*. After 120 minutes of exposure time, 90% of repellency was observed. After 15, 30, and 60 Min. of exposure time, 46%, 58%, and 76% of repellencies were observed, respectively. *Cl. ternatea* extracts exhibited 86% of repellency after 120 Min. of exposure. Repellency of 38%, 50%, and 68% were showed by the same extracts after 15, 30, and 60 Min. of exposure. Extracts of *C. pulcherrima* exhibited the highest repellency of 80% after 120 Min. of exposure. The same extracts showed repellency of 30%, 42%, and 62% after 15, 30, and 60 Min. of exposure time, respectively. Class -V repellency was exhibited by *Br.*

Pinnatum and *Cl. ternatea* extracts after 120 Min. of exposure. The same extracts exhibited Class-IV repellency after 60 Min. of exposure along with *C. pulcherrima* extracts after 60 and 120 Min. of exposure. After 15 Min. of exposure, *C. pulcherrima* and *Cl. ternatea* extracts exhibited Class-II repellency while, Class-III repellency was showed by *Br. Pinnatum* extracts.

Contact Toxicity Bioassay results of the three extracts showed concentration dependent toxicity. Highest toxicity of all the three extracts was exhibited by *Br. Pinnatum*. With 400 ppm 86.55 ± 0.70 mortality percentage was obtained by *Br. Pinnatum* extracts. Mortality percentages of 26.05 ± 0.59, 44.54 ± 0.70, and 64.71 ± 0.69 were showed by the same extracts with 100, 200, and 300 ppm concentrations. Probit Regression analysis results of *Br. pinnatum* extracts (Figure 5) indicated that LC50 and LC 90 values are 226.55 and 415.41 ppm, respectively. With slight decrease in the efficacy, *Cl. ternatea* extracts followed *Br. Pinnatum* extracts by exhibiting Mortality percentages of 19.83 ± 1.00, 31.90 ± 0.85, 56.90 ± 0.87, and 79.31 ± 0.89 with 100, 200, 300, and 400 ppm concentrations. Probit Regression analysis results of *Cl. ternatea* extracts (Figure 4) indicated that LC50 and LC 90 values are 247.56 and 451.80 ppm, respectively. The lowest toxicity of the three extracts was showed by *C. pulcherrima* extracts. At 400 ppm concentration, they delivered 72.17 ± 0.94 mortality only. At 300 ppm concentration also, they failed to showed 50% mortality (48.70 ± 0.88). Probit Regression analysis results of *C. pulcherrima* extracts (Figure 3) indicated that LC50 and LC 90 values are 260.92 and 485.26 ppm, respectively.

Table 1: Identified secondary metabolites in the selected plants' methanolic leaf extracts. Absent: -; Slightly Present: +; Moderately present: ++; Heavily present: +++

Extracts	Alkaloids	Flavonoids	Saponins	Terpenoids	Polyphenols	Glycosides
<i>C. limon</i>	+++	++	+	++	++	+
<i>A. vera</i>	++	++	+	+	++	-

Table 2: Repellent bioassay results of the tested extracts

Extract	Exposure Time (Minutes)	Mean Repellency (%)	Repellency Class
<i>Caesalpinia pulcherrima</i>	15	30	II
	30	42	III
	60	62	IV
	120	80	IV
<i>Clitoria ternatea</i>	15	38	II
	30	50	III
	60	68	IV
	120	86	V
<i>Bryophyllum pinnatum</i>	15	46	III
	30	58	III
	60	76	IV
	120	90	V

Table 3: Contact toxicity bioassay results of the test extracts against the adults of *S. oryzae* (Mortality percentages ± Standard Deviation after 72 Hrs).

Conc. In ppm	<i>C. pulcherrima</i>	<i>Cl. ternatea</i>	<i>Br. Pinnatum</i>
0	0 ± 0.50	0 ± 0.49	0 ± 0.44
100	14.78 ± 0.93	19.83 ± 1.00	26.05 ± 0.59
200	25.22 ± 0.98	31.90 ± 0.85	44.54 ± 0.70
300	48.70 ± 0.88	56.90 ± 0.87	64.71 ± 0.69
400	72.17 ± 0.94	79.31 ± 0.89	86.55 ± 0.70

Table 4: Probit analysis results of contact toxicity bioassays of the test extracts against the adults of *S. oryzae* after 72 Hrs. LC50 – 50% Lethal Concentration; LC90 – 90% Lethal Concentration- 95% CL – 95% Confidence Limits; LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

Extract	LC50	LC50 95% Confidence limits		LC90	LC 90 95% Confidence limits	
		LCL	UCL		LCL	UCL
<i>C. pulcherrima</i>	260.92	260.79	261.15	485.26	485.13	485.49
<i>Cl. ternatea</i>	247.56	247.41	241.80	451.96	451.80	452.19
<i>Br. pinnatum</i>	226.55	226.36	226.78	415.41	415.22	415.64

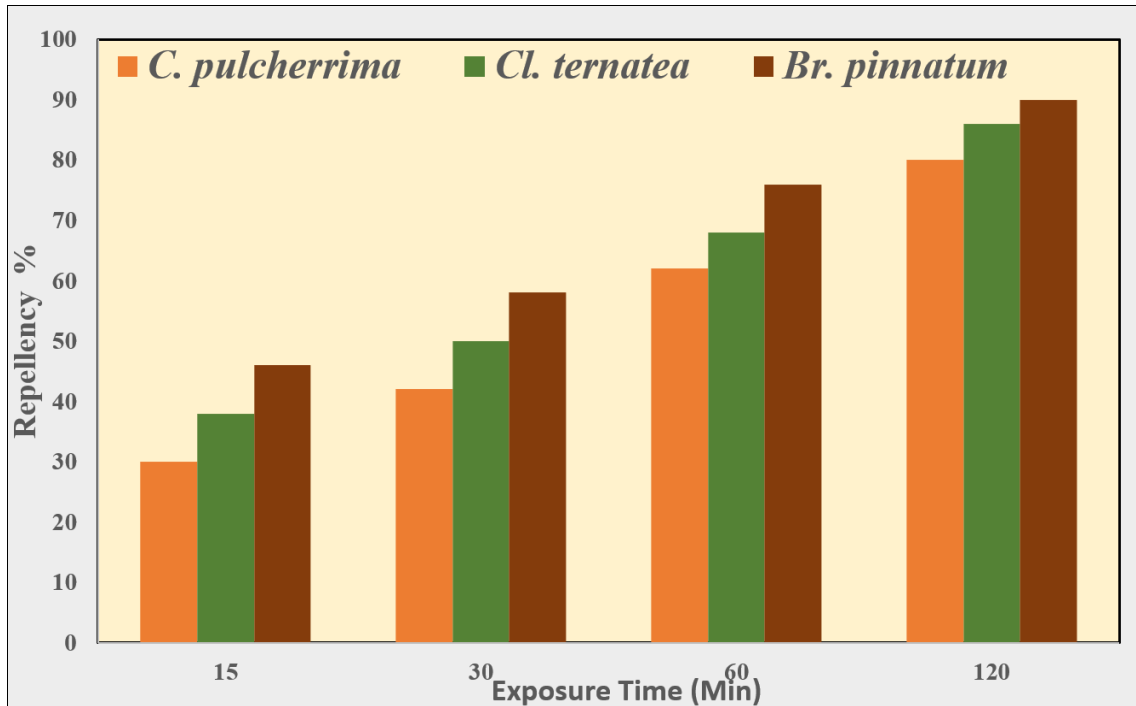


Fig 1: Repellent efficacy of crude methanolic leaf extracts of *C. pulcherrima*, *Cl. Ternatea*, and *Br. pinnatum* against the adult stages of *S. oryzae*.

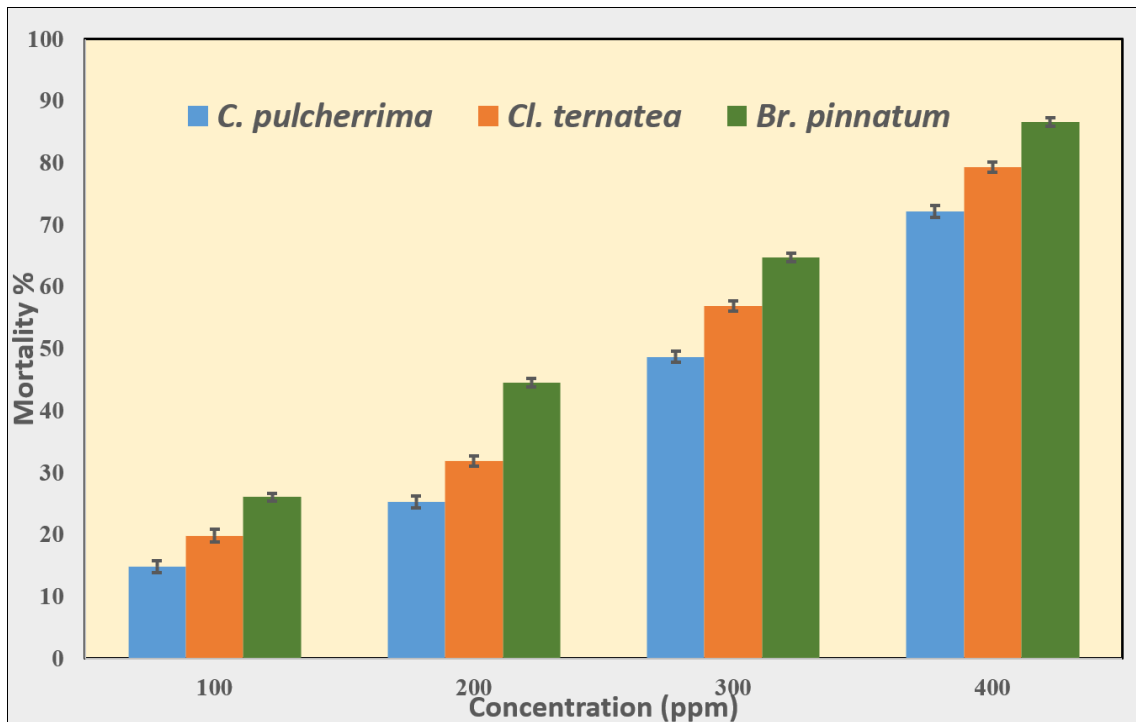


Fig 2: Contact toxicity efficacy of crude methanolic leaf extracts of *C. pulcherrima*, *Cl. Ternatea*, and *Br. pinnatum* against the adult stages of *S. oryzae*.

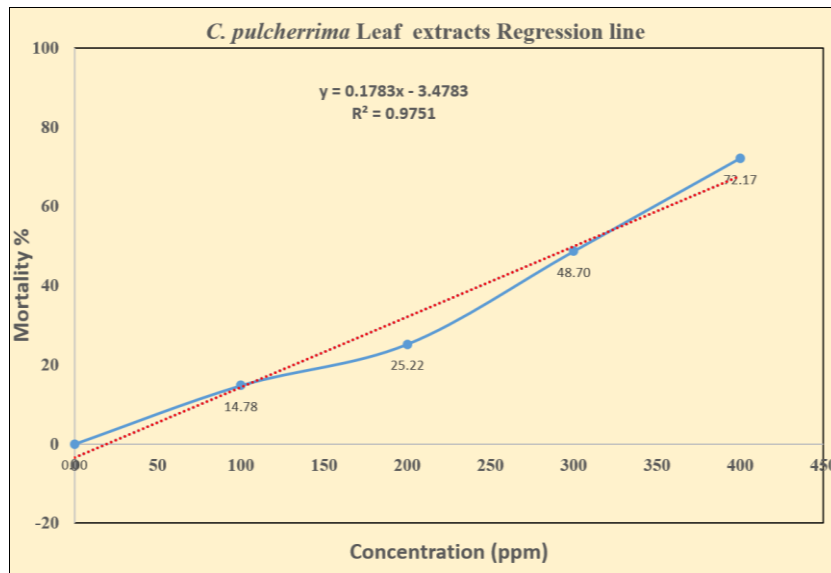


Fig 3: Regression analysis of Contact toxicity bioassay of crude methanolic leaf extracts of *C. pulcherrima* against the adult stages of *S. oryzae*.

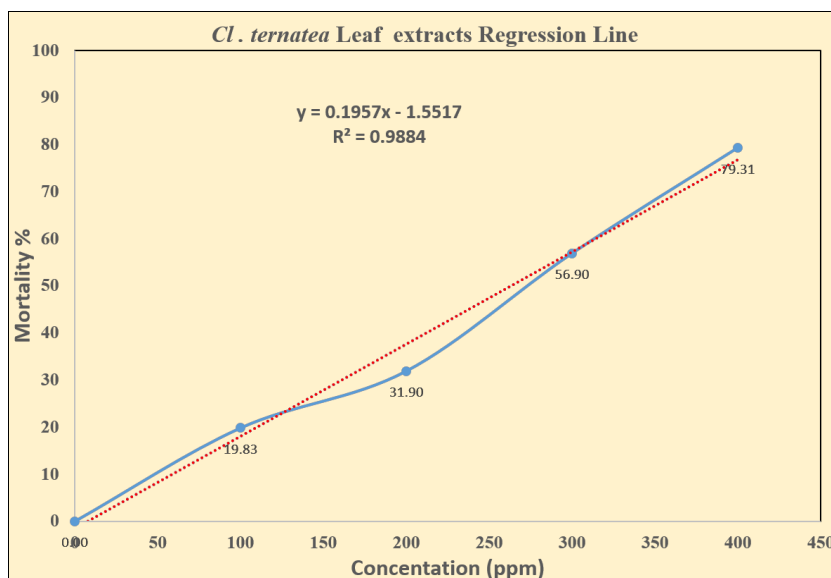


Fig 4: Regression analysis of Contact toxicity bioassay of crude methanolic leaf extracts of *Cl. ternatea* against the adult stages of *S. oryzae*.

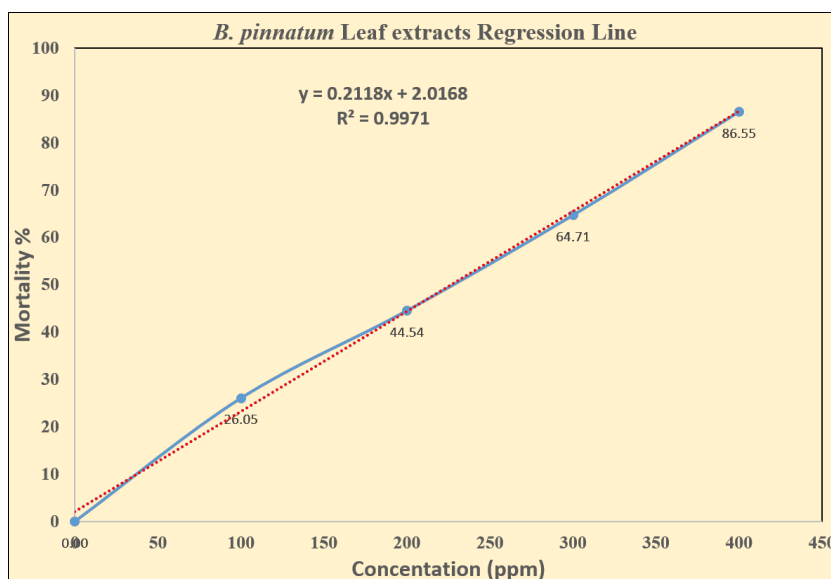


Fig 5: Regression analysis of Contact toxicity bioassay of crude methanolic leaf extracts of *Br. pinnatum* against the adult stages of *S. oryzae*.

Discussion

The present study revealed the repellent and contact toxicity efficacy of the crude methanolic leaf extracts of *C. pulcherrima*, *Cl. ternatea*, and *Br. pinnatum*. All the three extracts showed effective repellency and toxicity. Comparably, *Br. pinnatum* extracts performed better than the remaining two test compounds. Phytochemical analysis of the three extracts revealed the presence of alkaloids, flavonoids, terpenoids, phenols, and saponins which are well-known for their biological activities.

The results of the present study are supported by previous studies. Rahman, *et al.*, (2021) [21] evaluated the pesticidal activity of Sundarban mangrove plants - *Aegiceras corniculatum*, *Excoecaria agallocha*, and *Heritiera fomes*. The methanolic bark extracts of these plants against *S. oryzae* resulted in 80-100% mortality of the pests. Further experiments revealed that the 4th fraction of Solid Phase Extraction (SPE4) fraction showed activity against *S. oryzae*, with lethal concentration 50% (LC50) values of 0.5, 1.0, and 1.5 mg/disc for *A. corniculatum*, *E. agallocha*, and *H. fomes*, respectively.

In another study, Rani, *et al.*, (2019) [23] investigated the toxicity, repellent efficacy of several plant extracts against *S. oryzae* adults. They reported that a concentration of 3% exhibited the highest mortality rate against *S. oryzae*, with 100% mortality observed. The extract from custard apple leaves also showed significant pesticidal effects, resulting in 96.6% mortality of the target insect. In terms of repellency, the sweet flag rhizome extract demonstrated the highest repellent activity, with an 80.00% repellency rate. This was followed by the extract from dried chilies, which exhibited a repellency rate of 76.66%. Furthermore, the sweet flag rhizome extract displayed the highest fumigant toxicity action, causing 95.00% mortality of *S. oryzae* 72 hours after treatment.

The presence of various phytochemicals suggests that these compounds contribute to the observed repellent and toxic properties. Using natural plant extracts as alternatives to synthetic insecticides is a promising approach for sustainable pest management in stored grains.

Conclusion

This study examined the repellent and contact toxicity effects of crude methanolic leaf extracts from *Caesalpinia pulcherrima*, *Clitoria ternatea*, and *Bryophyllum pinnatum* against the rice weevil, *Sitophilus oryzae*. The results demonstrate the potential of these plant extracts as natural alternatives for controlling rice weevil infestations in stored grains. The extracts showed significant repellent activity, with *Bryophyllum pinnatum* being the most effective, followed by *Clitoria ternatea* and *Caesalpinia pulcherrima*. They also exhibited concentration-dependent toxicity, with *Bryophyllum pinnatum* being the most toxic, followed by *Clitoria ternatea* and *Caesalpinia pulcherrima*. Further research is needed to identify the specific bioactive compounds, optimize formulation techniques, and evaluate long-term efficacy in real-world storage conditions.

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