Biochemical Parameters and Digestive Enzymatic Activity of Ethanolic Leaf Extract of *Cocculus hirsutus* (L.) W. Theob. against the Pest *Rhynchophorus ferrugineus*

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Graphical Abstract:



Abstract

The red palm weevil (RPW), *Rhynchophorus ferrugineus*, is a highly destructive pest that affects more than 40 species of palm worldwide, including coconut plantations in India. The larvae feed on internal tissues and sap, causing severe damage, while their concealed life cycle makes early detection difficult. Although chemical and pheromone-based control methods are commonly used, there is limited research on eco-friendly alternatives. In the present study, we evaluated the bioefficacy of *Cocculus hirsutus* leaf ethanolic extract against RPW. We further assessed the impact of the plant extract on the biochemical constituents and digestive enzyme activities in treated larvae. Our findings suggest the potential of plant-based bio-products as an environmentally sustainable approach for managing RPW infestations and reducing reliance on chemical pesticides.

Keywords

Bio chemical, *Cocculus hirsutus,* Digestive enzymes, *Rhynchophorus ferrugineus,* pest

Introduction

The red palm weevil (RPW; *Rhynchophorus ferrugineus*) is one of the most destructive pests of palm species worldwide (Habood *et al.* 2022). Native to Southeast Asia, it has rapidly expanded its distribution and now poses a serious threat to economically important palms such as date palm, canary Island date palm, coconut palm, oil palm and other palm species also (Dembilio and Jaques 2015). The larval stage causes the most severe damage by feeding internally on palm tissues, often without visible early symptoms. This concealed feeding behavior makes early detection difficult, leading to sudden palm collapse. Common early signs include sap oozing, frass accumulation, and crown weakening. Management strategies include field sanitation, wound management, pheromone-based monitoring, and insecticide applications (Faleiro 2006; Dembilio and Jacas 2011; Al-Dosary *et al*. 2016). Botanical pesticides are emerging as promising components of integrated pest management programs against this devastating pest. Botanical pesticides are emerging as promising components of integrated pest management programs against this devastating pest.

Over millions of years, plants have evolved complex chemical defense mechanisms to protect themselves from herbivorous insects. These defenses include the production of diverse bioactive secondary metabolites with phototoxic, antibacterial, and antifeedant properties (Sathyananth *et al*. 2024). Botanical pesticides are derived from whole plants or specific plant parts using solvents, such as water, ethanol, or acetone, to extract active compounds (Dalavayi *et al*. 2021). These plant-derived secondary metabolites function as natural insecticides and may act through direct toxicity, anti-xenosis, and indirect defense mechanisms. By disrupting insect feeding, growth, development, digestion, and reproduction, phytochemicals, such as alkaloids, terpenoids, phenolics, and flavonoids, provide an eco-friendly and sustainable alternative for pest management (Isman 2006; Pavela and Benelli 2016; Isman 2020). Therefore, this study focused on using the leaf extract of *Cocculus hirsutus* (L.) treated with RPW, and enzymatic assays were performed.

Cocculus hirsutus (L.) is a medicinal climbing plant belonging to the family Menispermaceae, commonly known as "broom creeper" and locally called "Kattu kodi" in Tamil (Kumar *et al*. 2011). It is widely distributed in India and other parts of South Asia, where it is traditionally used to treat rheumatism, skin diseases, dyspepsia, gonorrhea, and other ailments (Thavamani *et al*. 2013; Singh *et al*. 2015; Nisha *et al*. 2023). The plant is rich in bioactive secondary metabolites, such as alkaloids (hirsutine, magnoflorine), flavonoids (quercetin, kaempferol), phenolics, tannins, saponins, and terpenoids. These phytochemicals contribute to its diverse pharmacological properties, including diuretic, analgesic, anti-inflammatory, hypotensive, and anticancerous activities. (Kirtikar and Basu 2005; Warrier *et al*, 1996; Harborne 1998; Isman 2006) Owing to its strong antimicrobial and insecticidal potential, *C. hirsutus* is increasingly explored as a promising botanical pesticide and natural therapeutic agent.

Materials and methods

Collection and Identification Plant

The plant was identified and selected from the area of Semmedu, located in Coimbatore, Tamil Nadu, India. It was authenticated by the Botanical Survey of India (BSI), Tamil Nadu Agricultural University Campus, Coimbatore, Tamil Nadu, India. First, the leaves were collected and washed with water and then shade-dried. After a week, the dried leaves were ground using a

mixture jar. The powdered sample was then extracted using ethanol in a Soxhlet apparatus. After extraction, the samples were used for further analysis.

Culture of Red palm weevil

For experimental purposes, the RPW culture was maintained under laboratory conditions at a temperature of 36±37 °C.

Experimental Analysis

For analysis, *C. hirsutus*-infected RPWs and control grubs were homogenized with 2 mL of 5% trichloroacetic acid and centrifuged for 20 min at 10,000 rpm at 4 °C.

Biochemical Studies

Estimation of protein

Bradford (1976) method, bovine serum albumin was used as the reference to measure the protein content of control and infected RPW grubs that were infected with *C. hirsutus* ethanol extract. 500mg of the sample was homogenised in 2ml of 8% trichloroacetic acid buffer solution and centrifuged. The supernatant was used for protein estimation. The optical density was read at 530 nm using a spectrophotometer. The results are presented in µg/mL protein.

Estimation of carbohydrates

This method was carried out as described by Hedge and Hofreiter (1962). The carbohydrate content of the control and infected RPW grubs was determined using glucose as the standard. Next, 500 mg of the sample was homogenized in 2 mL of 8% trichloroacetic acid buffer solution and centrifuged. The supernatant was used for carbohydrate estimation. The optical density was read at 620 nm using a spectrophotometer. The results are presented in µg/mL.

Estimation of Lipids

The Folch (1957) method was used to determine the lipid content of the control and infected RPW grubs using olive oil as the standard. For the sampling process, the supernatant was left undisturbed for 10 min after 1 mL of a chloroform: methanol combination and 1 mL of 0.9% NaCl was added. Next, 5 mL of 2% phosphovanillin was added to each test tube after 2 mL of

concentrated sulfuric acid was added, cooked for 20 min in a hot water bath, cooled, and left to stand for 10 min. Absorbance was measured at 540 nm using a UV spectrophotometer.

Digestive Enzymes

Determination of total protease enzyme activity

Kunitz (2017) by carried out this method. Briefly, 250 µL of the substrate (1 % caesin) solution, 250 µL of 0.1 M phosphate buffer (pH 7.6), and 100 µL of the control and *C. hirsutus* infected RPW grub extract in a test tube. The tubes were continuously shaken and incubated at 37 °C for 1 h. After 1 h, stop the reaction by adding 600 µl of 5% trichloroacetic acid and keep it on ice for 1h. After 1 h, the sample was centrifuged at 4000 rpm for 10 min. The supernatant was used for the determination of enzyme activity, and it was read at 280 nm.

Determination of amylase activity

Amylase enzyme activity was assayed as described by Bernfeld (1995). In the test tubes, 1 mL of 1% starch solution (substrate), 1 mL of 0.1 M phosphate buffer (pH 7.0), 1 mL of 1% NaCl, and 1 mL of the control and *C. hirsutus*-infected RPW grub extract sample were added. The test tubes were incubated for 1 h at 37 °C. After 1 h, the reaction was stopped by adding 0.5 mL of 3,5-dinitrosalicylic acid. Note the absorbance at 540 nm.

Determination of lipase enzyme activity

This method was developed by Stoytcheva *et al.* 2012. Taken 500 µl of the control and *C. hirsutus* infected RPW grub sample in the test tubes. Incubate at 100 ˚C for 5-10 minutes. Subsequently, 1000 µL of PVA (emulsion) and 500 µL of McIlvaine buffer were added to the tubes. The tubes were then incubated with constant shaking for 4 h at 37 ˚C and then 3 mL of alcohol: acetone (1:1) and 5 drops of phenolphthalein (1% in ethanol) were added. As the pH drops by about 0.2 units, add 0.1 N NaOH to bring the pH back to 7.0. Titrate for a 30-minute period and note the volume of NaOH consumed.

Determination of chitinase enzyme activity

The assay was performed following standard protocols described by Boller and Mauch (1988) and Miller (1959). They mixed 0.5 mL of colloidal suspension, 0.5 mL of control, and an infected RPW grub sample, and 1 mL of sodium acetate buffer in test tubes. The mixture was then

incubated at 37 ˚C for 1 h. The reaction was stopped by adding 1 mL of dinitrosalycylic acid (DNS). Next, the mixture was heated in a boiling water bath for 5–10 min. The tubes were cooled to room temperature. Absorbance was measured at 540 nm using a spectrophotometer.

Results

Mortality of RPW Grubs Treated with *C. hirsutus*

The mortality of RPW grubs increased progressively with increasing *C. hirsutus* concentration. The lowest mortality rate (40 ± 0%) was observed in the control treatment. A moderate increase in mortality was recorded at the 4% concentration (60 ± 0%), whereas the 6% concentration resulted in 70 ± 14% mortality, showing some variation between replicates. Higher concentrations (8% and 10 %) exhibited greater efficacy, causing 80 ± 0% and 100 ± 0% mortality, respectively. The highest mortality was achieved at 10%, indicating the complete susceptibility of RPW grubs at this concentration.

Table 1: Mortality of grubs treated with *C. hirsutus*

Concentration	Rep 1 Mortality (%)	Rep 2 Mortality (%)	Mean ± SD
Control	40	40	**40 ± 0^{a}**
4%	60	60	**60 ± 0^{ab}**
6%	80	60	**70 ± 14^{b}**
8%	80	80	**80 ± 0^{bc}**
10%	100	100	**100 ± 0^{c}**

(Values are expressed as mean ± SD (n = 2). Means sharing at least one common superscript letter are not significantly different at p ≤ 0.05 according to Tukey HSD test)

Biochemical parameters

Protein

The highest protein content was observed in the control group (1.59 ± 0.09 µg/mL). A gradual decline in protein levels was noted with increasing concentration, with values of 1.30 ± 0.08, 0.97 ± 0.03, 0.72 ± 0.02, and 0.66 ± 0.03 µg/mL at 4%, 6%, 8%, and 10%, respectively.

Table 2: Effect of *C. hirsutus* extract on protein content in RPW (µg/mL)

Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean ± SD**
Control	1.69	1.52	1.56	**1.59 ± 0.09^{d}**
4%	1.39	1.28	1.24	**1.30 ± 0.08^{c}**
6%	1	0.96	0.94	**0.97 ± 0.03^{b}**
8%	0.74	0.72	0.7	**0.72 ± 0.02^{a}**
10%	0.69	0.66	0.64	**0.66 ± 0.03^{a}**

(Values are expressed as Mean ± SD (n = 3). Means sharing the same superscript letter are not significantly different at p ≤ 0.05 according to Tukey's HSD test)

Carbohydrate

The highest carbohydrate content was observed in the control group (1.12 ± 0.36 µg/mL). Among the treated groups, carbohydrate levels showed a general reduction compared to those in the control. The lowest value was recorded at a 4% concentration (0.59 ± 0.08 µg/mL), followed by 10% (0.64 ± 0.08 µg/mL) and 8% (0.72 ± 0.03 µg/mL) concentrations, while a comparatively higher value was observed at a 6% concentration (0.86 ± 0.06 µg/mL).

Table 3: Effect of *C. hirsutus* extract on carbohydrate content in RPW (µg/mL)

Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean ± SD**
Control	1.09	1.5	0.77	**1.12 ± 0.36^{b}**
4%	0.49	0.65	0.63	**0.59 ± 0.08^{a}**
6%	0.8	0.88	0.92	**0.86 ± 0.06^{ab}**

8%	0.74	0.68	0.74	**0.72 ± 0.03^{ab}**
10%	0.62	0.74	0.58	**0.64 ± 0.08^{a}**

(Values are expressed as Mean ± SD (n = 3). Means sharing the same superscript letter are not significantly different at p ≤ 0.05 according to Tukey's HSD test)

Lipid

The highest lipid content was recorded in the control group (1.07 ± 0.52 μg mL^{-1}). All treated groups exhibited reduced lipid content compared to the control. Lipid levels decreased to 0.71 ± 0.03, 0.65 ± 0.03, and 0.53 ± 0.03 μg mL^{-1} at 4%, 6%, and 8% concentrations, respectively, while a similar value (0.65 ± 0.03 μg mL^{-1}) was observed at 10% concentration.

Table 4: Effect of *C. hirsutus* extract on lipid content in RPW (µg/mL)

Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean ± SD**
Control	1.6	0.56	1.05	**1.07 ± 0.52^{b}**
4%	0.74	0.68	0.72	**0.71 ± 0.03^{a}**
6%	0.68	0.62	0.66	**0.65 ± 0.03^{a}**
8%	0.56	0.5	0.54	**0.53 ± 0.03^{a}**
10%	0.68	0.62	0.65	**0.65 ± 0.03^{a}**

(Values are expressed as mean ± SD (n = 3).) Means sharing the same superscript letter are not significantly different at p ≤ 0.05 according to Tukey's HSD test)

Digestive enzymatic activity

Protease activity

The highest protease activity was observed in the control group (1.16 ± 0.00 µg/mL). In the treated groups, the activity showed a marked decline compared to that in the control. Protease activity was recorded as 0.09 ± 0.00, 0.07 ± 0.02, 0.03 ± 0.00, and 0.03 ± 0.01 µg/mL at 6%, 4%, 8%, and 10% concentrations, respectively.

Table 5: Effect of *C. hirsutus* extract on protease content in RPW (µg/mL)

Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean ± SD**
Control	1.15	1.16	1.16	**1.16 ± 0.00^{d}**
4%	0.04	0.08	0.1	**0.07 ± 0.02^{bc}**
6%	0.09	0.08	0.09	**0.09 ± 0.00^{c}**
8%	0.03	0.03	0.03	**0.03 ± 0.00^{a}**
10%	0.03	0.02	0.05	**0.03 ± 0.01^{ab}**

(Values are expressed as mean ± SD (n = 3).) Means sharing the same superscript letter are not significantly different at p ≤ 0.05 according to Tukey's HSD test)

Amylase

The highest amylase activity was recorded in the control group (1.20 ± 0.00 µg/mL). In contrast, all treated groups showed a marked reduction in enzyme activity. Amylase activity decreased to 0.04 ± 0.03, 0.07 ± 0.01, and 0.05 ± 0.03 µg/mL at 4%, 6%, and 8% concentrations, respectively. However, a relatively higher activity (0.13 ± 0.00 µg/mL) was observed at 10% concentration compared to the other treated groups.

Table 6: Effect of *C. hirsutus* extract on amylase content in RPW (µg/mL)

Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean ± SD**
Control	1.2	1.2	1.2	**1.20 ± 0.00^{c}**
4%	0.03	0.07	0.02	**0.04 ± 0.03^{a}**
6%	0.07	0.08	0.07	**0.07 ± 0.01^{a}**
8%	0.03	0.09	0.04	**0.05 ± 0.03^{a}**
10%	0.13	0.13	0.13	**0.13 ± 0.00^{b}**

(Values are expressed as mean ± SD (n = 3).) Means sharing the same superscript letter are not significantly different at p ≤ 0.05 according to Tukey's HSD test)

Lipase

The highest lipase activity was observed in the control group (1.63 ± 0.10 µg/mL). In contrast, all treated groups exhibited reduced enzyme activity, with values ranging from 0.67 ± 0.03 to 0.89 ± 0.12 µg/mL.

Table 7: Effect of *C. hirsutus* extract on lipase content in RPW (µg/mL)

Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean ± SD**
Control	1.69	1.52	1.69	**1.63 ± 0.10[b]**
4%	0.56	1.52	0.43	**0.84 ± 0.60[a]**
6%	0.58	1.15	0.6	**0.78 ± 0.32[a]**
8%	0.69	0.64	0.69	**0.67 ± 0.03[a]**
10%	0.92	1	0.76	**0.89 ± 0.12[a]**

(Values are expressed as Mean ± SD (n = 3). Means sharing the same superscript letter are not significantly different at $p \leq 0.05$ according to Tukey's HSD test)

Chitinase

The highest activity was observed in the control group (1.69 µg/mL). Among the treated groups, the activity ranged from 0.92 to 1.39 µg/mL. The lowest activity was recorded at a 4% concentration, whereas a relatively higher activity was observed at an 8% concentration.

Table 8: Effect of *C. hirsutus* extract on chitinase content in RPW (µg/mL)

Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean ± SD**
Control	1.69	1.69	1.69	**1.69 ± 0.00**
4%	0.92	0.92	0.92	**0.92 ± 0.00**
6%	1.3	1.3	1.3	**1.30 ± 0.00**
8%	1.39	1.39	1.39	**1.39 ± 0.00**
10%	1.09	1.09	1.09	**1.09 ± 0.00**

(Values are expressed as Mean ± SD (n = 3). No variation was observed among replicates)

Discussion

Every year, insect pests damage and destroy nearly one-fifth of the world's agricultural output. Researchers have been challenged by insect predation in agriculture to investigate novel, environmentally friendly natural insecticides. Worldwide, insect pests cause crop losses of approximately US$17.7 billion annually. In contemporary agriculture, the use of pesticides is essential. Pesticides are thought to be one of the main contributors to environmental contamination. Pesticides destroy beneficial micro-ecosystems in addition to contaminating land and water resources (Oerke 2006).

Botanical pesticides, known as botanicals, are derived from complete plants or specific plant parts. The chemically active element dissolves in the solvents used to extract it from the plant or plant part. At present, botanical pesticides are employed in agriculture to control insects and other pests instead of synthetic chemical pesticides (Pavela and Benelli 2016). Dalavayi *et al.* (2021) state in their study that over the past 25 years, hundreds of secondary metabolites or phytochemicals that demonstrate toxic or repulsive effects of insect pest feeding have been described and documented in the scientific literature. Hence, secondary metabolites of plants play a crucial role in pest control. This study aimed to control the pest RPW using the *C. hirsutus* leaf ethanol extract.

The chemical makeup of *C. hirsutus* was unknown for the purpose of researching its pharmacological effects, despite the fact that it was widely used as a traditional medicine. Alkaloids identified during preliminary and phytochemical screening, isolation, and characterization experiments included trilobine, isotrilobine, coclaurine, and magnoflorine. Additional compounds, such as beta-sitosterol, ginnol, and the mono-methyl ether of inositol, were also present. Three flavonoids, quercetin, liquiritin, and rutin, were present in the leaves. The plant extract of the entire plant also contained hirsutol, a triterpene derivative. When the aerial parts were extracted, beta-sitosterol and 28-acetyl botulin were identified. Numerous events have occurred, including studies that describe the extracts' preliminary phytochemical testing and show the presence of carbohydrates, steroids, alkaloids, glycosides, flavonoids, tannins, and saponins (Nisha *et al*. 2023).

Al *et al*. 2016tested the efficacy of *Azadirachta indica* extracts against RPW stages and used five individuals of full-grown larvae. The results show laboratory toxicity tests revealed average mortality rates of 88.3±1.3 and 84.3±0.4 % for larval and adult stages at 6 g/mL

concentration, respectively, whereas mortality rates were 79.6±1.2 and 74.5±0.7 % on average for larval and adult stages, respectively, in the case of 3 gm/ml concentration. The lowest average mortality rates were 77.9±1.3 and 72.3±0.9 % for larval and adult 3stages, respectively, with the treatment of the concentration 1.5 g/mL. whereas the lowest larval and adult mortalities (66.7 and 57.3 %) were gained by the neem petroleum ether extract. Through laboratory screening tests, RPW larvae showed higher susceptibility to the applied treatments than adult stages. Bioassay experiments indicated that the mortality levels of *R. ferrugineus* stages subjected to the Ethanolic neem extracts concentrations, especially with the titer of 6 gm /ml proved to be more virulent against RPW stages than those of the petroleum ether neem extract. The above study states that two extracts of *A. indica* were used to control the pest of RPW, and the highest results were noted for the ethanolic extract at a concentration of 6 g/mL. Likewise, the present investigation also treated the pest with different concentrations of *C. hirsutus* leaf ethanol extract, and the grub mortality was observed at the highest concentration of 10% at 14 h and followed by 16 hours an 8% dosage. This may be due to the presence of the richest volatile compounds in the plant, which affect the physiological actions of the insect and lead to grub death.

Quantification of total protein is crucial in physiological investigations because proteins are essential biochemical components required for growth, development, and the performance of essential functions in organisms. The present study also involved biochemical analysis of proteins, carbohydrates, and lipids. Protein content in the treated grub of RPW decreased compared to the control in the present investigation. Protein degradation to provide energy or protein conversion to amino acids could explain the decline (Habood *et al.* 2022).

Glucose, protein, TAG, and cholesterol levels decreased at the highest insecticidal dose (10% w/v) owing to the deterrent and antinutritional properties of the extract. Insects may have gradually reduced their lipid and glycogen stores by eating less, which would have also hindered the production of proteins and steroid hormones, which are synthesized from cholesterol. The decreased absorption observed in the nutritional parameter trials is most likely the reason for the decrease in measured cholesterol levels because insects do not synthesize cholesterol, which is obtained from the diet or from symbiotic microbes. This may also explain our finding that compared to the grub control, all the infected showed decreased activity.

Studies evaluating sublethal treatments have reported a significant reduction in total carbohydrate and glycogen content in treated larvae compared with untreated controls, indicating disruption of carbohydrate metabolism and energy homeostasis (El-Sobki and Ali 2020; Diab *et al*. 2019). Such depletion is primarily attributed to feeding deterrence, impaired digestive efficiency, and increased metabolic demand for detoxification under chemical stress (Ghoneim *et al*. 2012; Senthil-Nathan 2013). Alterations in carbohydrate-metabolizing enzymes, including trehalase and invertase, have also been observed in treated larvae, further confirming interference with carbohydrate digestion and utilization pathways (El-Sobki and Ali 2020). Although direct studies employing botanical extracts against *R. ferrugineus* are limited, evidence from biopesticide and insecticide exposure clearly supports that carbohydrate depletion is a key biochemical consequence leading to reduced growth, weakened physiological condition, and eventual larval mortality (Diab *et al*. 2019). These findings are in accordance with reports on other coleopteran pests, where plant-derived compounds exert antifeedant and metabolic inhibitory effects, ultimately resulting in energy starvation and pest suppression (Senthil-Nathan *et al*. 2005).

In insects, lipid metabolism is crucial because lipids are stored as body fat and provide energy to regulate metabolic processes, including the synthesis of reserves and vital metabolites for the insect progeny, such as vitellogenins, during egg development (Arrese and Soulages, 2010; Nation 2015; Chapman, 2013**)**. Considering this statement, lipids are important for many needs, especially for vitellogenesis. Therefore, the results of the present investigation show a decreased level of lipids, which would have led to a delay in egg-laying.

Important digestive enzymes, such as proteases, amylases, and lipases, which are necessary for the digestion of proteins, carbohydrates, and fats, are significantly reduced in quantity when insect grubs are treated with plant-derived extracts. According to Senthil-Nathan *et al*. (2006) and Nathan *et al*. (2005), botanical insecticides disrupt the production and activity of these enzymes, which impairs the larvae's ability to absorb and digest food. When larvae were exposed to neem, Calotropis, Ocimum, and other medicinal plant extracts compared to untreated controls, quantitative enzyme assays revealed significant reductions in proteolytic, amylolytic, and lipolytic activities, indicating strong antinutritional effects (Senthilnathan 2013). Antifeedant activity, injury to the midgut epithelium, and direct suppression of enzyme secretion by plant secondary metabolites, such as alkaloids, tannins, flavonoids, and terpenoids, are the main causes of the decrease in digestive enzyme levels. Consequently, enzyme inhibition causes grubs to grow more

slowly, live longer as larvae, experience metabolic stress, and eventually die. All the above investigations reveal that enzyme inhibition is a key biochemical mechanism for plant extract-mediated insect control of both lepidopteran and coleopteran pests.

RPW grubs treated with *C. hirsutus* extract showed a significant decrease in lipase, amylase, and protease activities, indicating impaired digestive efficiency, which corroborates previous reports on botanical insecticides (Senthil Nathan *et al*. 2006; Nathan *et al*. 2005).

In insect larvae, chitinases are essential for cellular membrane integrity, midgut peritrophic membrane maintenance, and chitin turnover. According to quantitative biochemical research, botanical treatments, especially those based on neem and other plant secondary metabolites, inhibit chitin metabolism by lowering chitinase activity. This causes treated larvae to exhibit abnormal cuticle formation, defective molting, and growth inhibition (Schlüter and Bernt 2000; Nathan *et al*. 2008). Molecular and biochemical investigations have confirmed that the inhibition of chitinase-mediated pathways is a critical mechanism leading to molting failure and larval mortality, highlighting chitin metabolism as a major biochemical target of botanical insecticides (Retnakaran *et al*. 2001). In the present study, RPW grubs treated with *C. hirsutus* plant extract showed a significant reduction in chitinase activity compared to untreated controls. The decline in enzyme activity increased with concentration and exposure period, indicating dose-dependent biochemical disruption. These results corroborate earlier reports that botanical compounds suppress chitin metabolism and membrane-associated enzymes, ultimately leading to impaired molting and larval mortality.

The use of biopesticides in integrated pest management (IPM) has increased owing to the growing need for environmentally friendly agricultural practices. Biopesticides, which are derived from microbes, plants, or biological materials, target specific pests without endangering non-target species, making them safer and more environmentally friendly alternatives to synthetic pesticides. In IPM, biopesticides serve as a viable substitute that enhances soil health and reduces the need for chemical pesticides (Ashiq 2024). This helps to mitigate global warming, which is an urgent concern.

Conclusion

The *C. hirsutus* leaf extract showed insecticidal activity, which interfered with insect defense mechanisms, suppressed the immune system, and increased oxidative damage and cytotoxicity in the insect *R. ferrugineus* grub when treated, which may have caused its mortality.

As chemical and pheromone trap methods are highly recommended for controlling this pest, knowledge of natural pesticide awareness is lacking. Therefore, this study concludes that the pest RPW can be controlled using *C. hirsutus* leaf extraction as a green pesticide.

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