International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 11-04-2026, Accepted: 12-05-2026, Published: 27-05-2026
Volume 11, Issue 2, 2026, Page No. 302-308

Effect of an aqueous extract from *Murraya Koenigii* against the wheat storage pest, *Tribolium Castaneum*

Amandeep Kaur[1*], Shelly[1], Bindu Bala[1], Harpreet Kaur[2], Pardeep Kaur Sandhu[3]
[1] Department of Zoology, Sri Guru Teg Bahadur Khalsa College, Sri Anandpur Sahib, Punjab, India
[2] Department of Zoology, Government Mohindra College, Patiala, Punjab, India
[3] Department of Zoology, Mata Gujri College, Fatehgarh Sahib, Punjab, India

Abstract
Pest infestation is a primary factor contributing to decreased productivity in both field crops and stored grains. Although synthetic insecticides and pesticides are commonly used to mitigate economic losses, their application is associated with adverse effects on human health and the environment. In light of these concerns, the present study evaluated the insecticidal potential of an aqueous extract of *Murraya koenigii* against *Tribolium castaneum*. The extract was assessed for its adulticidal, larvicidal, residual, and repellent activities across five concentrations (2 ppm > 1.75 ppm > 1.5 ppm > 1.25 ppm > 1 ppm). Results indicated a significant dose-dependent efficacy in all tested parameters. The observed bioactivity is likely attributable to the presence of bioactive phytochemicals, including phenolic compounds such as rutin, quercetin-3-glycoside, myricetin, and quercetin, along with volatile constituents like β-caryophyllene, α-cedrene, α-copaene, β-cubebene, and germacrene D. These findings suggest that *M. koenigii* may serve as a promising botanical source for the development of eco-friendly insecticidal agents targeting *T. castaneum*, thereby offering a potential alternative to synthetic chemical pesticides.

Keywords: *Murraya koenigii, Tribolium castaneum, pest,* bioactive phytochemicals, repellent activities

Introduction

Postharvest losses due to insect pests are a major concern in agricultural systems, significantly impacting the productivity and quality of both field crops and stored grains (Anonymous, 1989). The term *pest*, derived from the Latin word *pestis* meaning plague, broadly refers to any organism—including insects—that damages crops, stored food products, or livestock. Storage insect pests are generally categorized into two types: primary pests, which infest whole, healthy grains (e.g., internal and external feeders), and secondary pests, which attack already damaged or broken grains. Prominent primary storage pests include *Sitophilus oryzae* (rice weevil), *Rhyzopertha dominica* (lesser grain borer), *Sitotroga cerealella* (Angoumois grain moth), *Callosobruchus chinensis* (pulse beetle), *Pachymeres gonagra* (tamarind beetle), *Cylas formicarius* (sweet potato beetle), *Phthorimaea operculella* (potato tuber moth), *Tribolium castaneum* (red flour beetle), and *Trogoderma granarium* (Khapra beetle). Secondary pests include *Oryzaephilus surinamensis* (saw-toothed grain beetle), *Latheticus oryzae* (long-headed flour beetle), *Cryptolestes minutus* (flat grain beetle), *Acarus siro* (grain mite), and *Corcyra cephalonica* (rice moth).

Among these, *Tribolium castaneum* is particularly detrimental to stored cereal products such as wheat flour, porridge oats, rice bran, and grain. As a cosmopolitan species, *T. castaneum* thrives in a wide range of environments, with optimal development occurring at temperatures around 35 °C and relative humidity levels of 60–80%. Its life cycle comprises four stages—egg, larva, pupa, and adult—with females capable of laying 300–400 eggs during a lifespan of 4–5 months. Both larval and adult stages inflict substantial damage to stored grains, primarily by consuming the endosperm and leaving behind only the seed coat. This renders the grain nutritionally unfit and unsuitable for human consumption. Additionally, the accumulation of larval excreta imparts a musty odor and further degrades the grain quality. Chemical insecticides, including malathion, fenitrothion, permethrin, deltamethrin, and cypermethrin, are routinely used to manage storage pests. Uncontrolled use of these chemicals causes great environmental hazard due to their persistent nature, increased risk of neuro toxic, carcinogenic, teratogenic and mutagenic effects in non-target animals, acute residual toxicity, ability to create hormonal imbalance, spermatotoxicity (Khatter 2011) [7].

In recent years, increasing concern over chemical residues, insect resistance, and environmental safety has accelerated research into non-chemical alternatives for stored-grain pest management. Physical and irradiation-based control techniques have emerged as promising, eco-friendly strategies capable of suppressing insect populations without compromising grain quality or consumer safety (Abdel Halim *et al*., 2025) [1].

In light of these issues, there is a growing interest in developing alternative pest control strategies that are effective, environmentally benign, and safe for human use. Botanical insecticides, particularly those derived from plant extracts, have emerged as promising candidates due to their biodegradability, target specificity, and lower ecological footprint. Various plant-derived substances, including oils and extracts obtained using solvents like methanol, ethanol, and hexane, have demonstrated repellent and insecticidal properties against stored grain pests.

Among physical control methods, gamma irradiation has been demonstrated to effectively suppress multiple life stages of stored-product insects, including eggs, larvae, pupae, and adults. Enhanced pest suppression has been reported when gamma irradiation is combined with biological control agents such as *Trichogramma* spp., indicating strong potential for integrated pest management programs in stored-grain ecosystems (Abdel Halim *et al*., 2025) [1].

Ultraviolet-C (UV-C) irradiation has also gained attention as an effective non-chemical approach for controlling storage pests. Recent studies have shown that UV-C exposure significantly reduces pest populations while maintaining seed viability and grain quality, highlighting its suitability as an environmentally friendly post-harvest treatment (Keszthelyi *et al*., 2024) [6]. In addition to irradiation, microwave-based technologies have shown considerable promise in stored-grain pest management. Microwave treatments have demonstrated high insect mortality rates while preserving grain quality and improving detection efficiency, making them suitable for rapid post-harvest disinfestation procedures (Miao *et al*., 2026; Tuychieva, 2025) [10, 15].

Several plant species have shown potential in this regard, including *Azadirachta indica* (neem), *Tamarindus indica* (tamarind), *Cucumis sativus* (cucumber), *Psidium guajava* (guava), *Artemisia vulgaris* (common mugwort), *Murraya koenigii* (curry leaf), *Prosopis juliflora* (kikar), *Matricaria chamomilla* (chamomile), and *Solanum sisymbriifolium* (red buffalo-bur).

Murraya koenigii, a member of the Rutaceae family, is native to Sri Lanka, India, and other parts of South Asia. Commonly known as the curry leaf tree, it is widely used in traditional medicine and culinary practices. The plant is rich in nutrients, including vitamins A, B, C, and E, and exhibits multiple bioactivities, such as antioxidant, antimicrobial, and insecticidal properties.

Morphologically, *M. koenigii* is a semi-deciduous, aromatic shrub with pinnate, gland-dotted, fragrant leaves. The phytochemical composition of the leaves includes significant amounts of moisture (63.2%), crude fiber, nitrogen, sugars, starch, fats, and various extractive substances. Prior research has reported the insecticidal efficacy of *M. koenigii* extracts against pests such as *Bemisia tabaci* and *Callosobruchus chinensis*.

Given its bioactive potential, the present study was undertaken to investigate the insecticidal and repellent activity of the aqueous extract of *M. koenigii* leaves against *T. castaneum*, a major pest of stored wheat. The aim is to evaluate the efficacy of this botanical extract in mitigating postharvest losses while promoting safer, eco-friendly pest control alternatives.

Materials and Methods

Study Area

Current study was carried out at Sri Guru Teg Bahadur Khalsa College Sri Anandpur Sahib, Punjab.

Rearing of culture of test insect

Tribolium castaneum beetles were cultured in infested wheat grains and reared at temperature range of 28± 2^0C and 65±5% RH at a dark place. After 2 weeks of egg laying, only the egg laden kernels were separated and dead and live adults were removed from the culture. The separated egg laden grains were cultured again. New broken wheat grains were taken, washed and dried properly in sunlight and placed in refrigerator to avoid the traces of any infestation. Glass jars were taken and sterilized before filling the jars with broken wheat grains. Fifty beetles were introduced into the jars. Jars were kept at a dark place with mentioned relative humidity. Humidity and temperature were monitored by hygrometer. It was observed that the broken grains facilitated the growth and multiplication of test insects. As the population of test insect increased inside the jars, fresh wheat was added into new jars and partial culture was transferred to them. In this way, Larvae and adults of *T. castaneum* were obtained from this culture.

Preparation of Plant extracts

The aqueous extract of *M. koenigii* was prepared by using its leaves. The leaves were dried and grinded then extract was prepared by using water as a first solvent. The extract was dried by heating upon water bath via a thin film evaporator till the solvent was completely evaporated and dry residue of plant was kept in airtight bottles in the refrigerator at 4^0C temperature until further use. Acetone was used for dilution of extract and to make solutions of different concentrations.

Study of Adulticidal/Larvicidal effect

Small containers were washed, dried and sterilized. 15 gms of broken wheat grains were taken in each container. The plant extract was added to the wheat by the help of micropipette and mixed well with a glass rod to ensure adequate coating of grains until the acetone was completely evaporated. 20 adults and 10 larvae of the test insect were released and the lid of containers was applied. The inside of container was covered by petroleum jelly just above the level of wheat to prevent insects from crawling on the sides. After 24 hours of treatment, mortality counts were made. The mortality range was found out arranging several preliminary trials to obtain mortality between 10-90%. Each extract was used at five different dose levels and replicated in three sets under laboratory conditions. Side by side control having untreated 15 gms of wheat containing 20adults and 10 larvae individually in two containers was also run in parallel.

Repellent Action

Transparent plastic box was taken and lined by Whatman filter paper. Filter paper was divided into two equal halves. One half of paper remained untreated and the other half was sprayed by 0.25% concentrated solution of plant extract. Then the filter paper was placed into the transparent plastic box. 20 test adults were introduced in the central area of the box, then the box was covered by a lid. Each treatment was performed thrice. After an hour, the adult insects were counted in treated and untreated area. A control was also run in parallel. As a result, even the lowest concentration was found effective to repel the adult *T. castaneum.*

Study of residual toxicity

100 gms broken wheat grains were taken and treated with aqueous extract of curry leaves. The wheat grains were treated well with extract to ensure the proper coating of. A dose with maximum adulticidal effect (2ppm) was used. Then the treated grains were stored into airtight containers. After treatment of the wheat, the test to analyze the residual toxicity was done after 12 hrs, 24 hrs, 48 hrs, and 72 hrs. For the test 15 gms of treated wheat was taken in a sterilized container. 20 adults of test insect were released into it and the lid was applied properly. Mortality count was made after 72 hours. Each test was run into three replications. Control test of untreated wheat was also run parallel.

Results and Discussion

In the present investigation, the insecticidal potential of the aqueous extract of *Murraya koenigii* was evaluated against the red flour beetle, *Tribolium castaneum*, under laboratory conditions. The study aimed to assess the adulticidal, larvicidal, residual, and repellent effects of various concentrations (1 ppm, 1.25 ppm, 1.5 ppm, 1.75 ppm, and 2 ppm) of the extract.

1. **Adulticidal and Larvicidal Activity**
Each experimental set consisted of 20 g of wheat grains treated with the aqueous extract, with three replicates per concentration. Ten larvae or adults were introduced into each culture tube, and mortality was recorded after 24 hours. A control group (untreated wheat) was maintained concurrently. The insecticidal activity showed a concentration-dependent pattern:

Toxicity trend
2 ppm > 1.75 ppm > 1.5 ppm > 1.25 ppm > 1 ppm
This pattern was consistent across both adult and larval stages, indicating the extract's broad-spectrum efficacy. The significant mortality observed at higher concentrations suggests that bioactive constituents present in *M. koenigii*, such as phenolic compounds (e.g., rutin, quercetin-3-glycoside, myricetin, quercetin) and volatile compounds (e.g., β-caryophyllene, α-cedrene, α-copaene, β-cubebene, germacrene D), likely contributed to the observed toxicity. These compounds are known to exhibit neurotoxic, growth-inhibitory, or digestive-disrupting effects in insects.

2. **Repellency Effect**
Repellency assays revealed that the aqueous extract effectively repelled adult *T. castaneum* even at low concentrations, with a trend similar to that of the toxicity data:

Repellency trend
2 ppm > 1.75 ppm > 1.5 ppm > 1.25 ppm > 1 ppm
This suggests that even sub-lethal concentrations can deter infestation, likely due to olfactory disruption caused by the plant's aromatic volatiles. Similar mechanisms have been documented in extracts from neem (*Azadirachta indica*), eucalyptus, citronella, and lemongrass, where active compounds like azadirachtin, citronellal, and eucalyptol alter sensory perception in pests (Upadhyay and Jaiswal, 2007; Tripathi *et al* 2009) [14].

3. **Residual Toxicity**
Residual efficacy was assessed by measuring mortality at 12, 24, 48, and 72 hours after treatment. The maximum residual impact was recorded at 12 hours post-treatment and gradually declined over time. These results underscore the rapid but potentially short-lived insecticidal action of aqueous extracts.
Recent studies suggest that integrating botanical insecticides with physical control techniques, such as irradiation or microwave treatments, may enhance long-term pest suppression while reducing dependence on synthetic chemicals. Irradiation technologies have also been successfully applied to stored-product insects infesting edible insect rearing systems, confirming their safety and broader applicability beyond conventional grain storage (Sileem *et al*., 2024) [12].
Factors such as volatility, environmental degradation, and extract composition may influence persistence. The insecticidal and repellent activity of *Murraya koenigii* observed in the present study aligns with findings from previous research involving various plant-based extracts.

Recent reviews further emphasize that integrating eco-friendly technologies—such as irradiation, microwave treatment, and advanced pest detection and monitoring systems—can substantially reduce post-harvest losses and reliance on chemical pesticides (Tamilarasan *et al*., 2025; Vadivambal & Jayas, 2021) [13, 17]. Advances in pest detection and monitoring technologies also support precision management approaches, enabling early infestation detection and targeted intervention in stored-grain systems (Gong *et al*., 2026) [5].

Neem (*Azadirachta indica*) extracts, particularly those containing azadirachtin, have been extensively documented for their potent effects on insect pests, including *T. castaneum*. Azadirachtin disrupts the hormonal balance of insects, leading to delayed moulting, reduced fecundity, and ultimately, mortality. Similarly, eucalyptus oil, which contains eucalyptol, has demonstrated strong fumigant and contact toxicity effects, causing neuromuscular disruptions in insects. Lemongrass oil, rich in citral, has been shown to act as both a toxicant and a repellent, significantly inhibiting feeding and reproductive behaviour. Capsaicin, the pungent compound in chili peppers, and allicin, the bioactive compound in garlic, have also been found to possess insecticidal properties by damaging the digestive tract and disrupting sensory functions in beetles. Amel *et al* (2014) [2] concluded ethanol extract of pomegranate peel has toxicity against red flour beetle and showed 56% larval mortality. Several plant oils and extracts have strong repellent properties. Compounds like citronella, eugenol, and cinnamaldehyde deter beetles from feeding or laying eggs on treated surfaces. Some extracts cause growth disruption by blocking molting or inhibiting the digestion of food, thus preventing the insect from completing its lifecycle. Higher concentrations of plant extracts tend to result in greater insecticidal activity, but formulations must also consider the stability and persistence of the compounds. For instance, essential oils like neem or eucalyptus oils may lose potency over time due to volatility. Padin *et al* (2013) [11] showed plants with methanol extract showed insecticidal activity. Temperature, humidity, and exposure to light can also affect the performance of plant extracts. Some plant-derived compounds are more effective under certain environmental conditions, such as high humidity or warm temperatures, which are typical in storage areas. Liska *et al* (2011) [8] tested bioactivity of 1,8-cineol, camphor and eugenol composition of essential oils from aromatic plants, to mitigate progeny in *Tribolium castaneum*. Compound 1,8-cineole, camphor and eugenol were applied at lowest concentration 120 micro l/350ml-1vol). The progeny ranged between174.25 to221,50 and higher concentration 600microl/350ml-1vol lowest impact appear in progeny of *T. Castaneum* had camphor (191.00) which was lower related to 1,8- cineole (72.25) and eugenol (112.00). This indicates camphor have no impact upon number of progenies. Vijay Kumar *et al* (2015) [18] determined biological activities of spices namely turmeric, chilly, coriander, fennel seeds, black pepper, ginger, fenugreek garlic and cumin against *Tribolium castaneum*. All spices showed significant effect on adult mortality. Toxic effect followed-black pepper> cumin> garlic> fennel seed>ginger>fenugreek>untreated control. Huang *et al* (1997) reported that nutmeg oil in concentration of

1.05g/100gm of wheat, totally suppressed F-1populationof *Tribolium castaneum*. Larvae were more susceptible than adults to contact toxicity. Extracts were obtained by steam distillation and tested for contact toxicity. Upadhyay and Ahmad (2011) [16] explained that besides high mortality of adult insects, reduced oviposition and hatching, essential oil of black pepper significantly suppressed survival of larvae and adults. Azevedo *et al* (2008) [3] treated Peanut seeds with neem oil concentration of 0.0, 0.5, 1.0, 1.5 and 2.0% (volume/seed mass) and mortality of *Tribolium castaneum* offspring number (larvae, pupae and adults) and number of punched seeds were evaluated in four stored period (30, 60, 90 and 120 days). The effect of neem on biological development was observed.

Wang *et al* (2009) [9] estimated the effects of monoterpenes of 3-carene, 1, 8-cineole, beta-pinene, terpinene and terpinolene as repellents, against *Tribolium castaneum* adults. Monoterpene of 1, 8-cineole in 20microLml-1 and beta pinene in 20 microLml-1 exhibited highest percent of repellency.

Mortality tests were conducted at intervals to analyse the impact of remaining residues left in the grains at different hours after treatment (HAT). Concentration of spice extracts giving 90% mortality were mixed with wheat grain (v/w) basis. With the help of acetone desired concentration of solution were made out of extract prepared. 10 gm of treated wheat taken and 10 adult *T. castaneum* were introduced. Mortality was recorded after 12, 24, 48 and 72 hr period of exposure. Treatment after 12hr gave maximum residual efficacy followed by 24, 48 and 72 hours after treatment. Devi and Devi (2013) [4] screened various spices against *Sitophilus oryzae*, a serious stored food grain pest. Bioefficacy of powders and hexane extracts were determined. Responses varied with spices, dosage and exposure time. Mace and pepper, at 1% level resulted total mortality by one week followed by nutmeg and clove with 100% mortality and cinnamon and star anise with 90% mortality at 5% concentration.1000 ppm showed insecticidal activity, with pepper extract recording 100% mortality by 5 days. Clove oil resulting in 92% mortality, 51.63% nutmeg, 66.6% cinnamon, 79% in case of mace. Hexane extracts of star anise, cinnamon and clove at 0.59 microliter/cm^2 on filter paper discs induced 100% mortality by 72 hour. Spices offered protection to wheat up to 9 months without affecting seed germination thereby showing promise as grain protectants. Meena *et al* (2015) [9] investigated that 0.5% dose of black pepper seed powder against *Rhyzopertha dominica* exhibited maximum adult mortality, 65% and 29.75% after 48hrs adults were released at 4 and 60 DAT respectively. Tripathi and Verma (2000) [14] evaluated the efficacy of powdered black pepper (*Piper nigrum*) against *Rhizopertha dominica*. Dose of 3g pepper per 100g wheat resulted in 100% mortality after 15 days, as did 2.5g after 25 days.

The bioactivity of plant extracts is attributed to a variety of mechanisms that affect insect physiology and behaviour. Certain compounds, such as pyrethrins and eucalyptol, act on the insect nervous system, disrupting synaptic transmission and causing paralysis. Others, like azadirachtin, interfere with hormonal regulation, delaying development and reproduction. Digestive inhibitors such as capsaicin and allicin cause severe gastrointestinal distress, leading to starvation and eventual death. Additionally, several plant volatiles exhibit strong olfactory effects that repel insects by interfering with their sensory receptors. However, the effectiveness of these compounds is influenced by environmental conditions, including temperature, humidity, and light exposure. Volatile oils, for example, may degrade or evaporate rapidly, reducing their residual efficacy. Therefore, while plant-based insecticides offer eco-friendly alternatives to synthetic chemicals, their practical application requires careful attention to formulation stability and environmental interactions.

Conclusion

The promising results of this study highlight the potential of *M. koenigii* aqueous extract as an effective biopesticide against *T. castaneum*. Its demonstrated adulticidal, larvicidal, and repellent activities suggest that it could serve as a natural alternative to chemical insecticides, contributing to more sustainable pest management practices. However, this study represents only an initial step toward commercial application. Further research is necessary to isolate and characterize the specific bioactive constituents responsible for the insecticidal effects. Additionally, it is important to evaluate the long-term stability of these extracts under real storage conditions, assess their safety for human health and non-target organisms, and optimize delivery methods for large-scale use. By addressing these aspects, the development of standardized, effective, and environmentally friendly grain protectants derived from *M. koenigii* and similar botanicals can be realized, reducing reliance on hazardous synthetic chemicals and mitigating the environmental impact of pest control strategies.

Table 1: Toxicity of aqueous extract against Adults of *Tribolium castaneum*

S. No.	Dose (ppm)	R1	R2	R3	Mean±SD
1	2ppm	8^{b}	7^{b}	7^{b}	$8.00±0.57^{b}$
2	1.75ppm	6^{b}	5^{b}	8^{b}	$6.30±1.52^{b}$
3	1.50ppm	5^{ab}	3^{ab}	6^{ab}	$4.60±1.52^{ab}$
4	1.25ppm	3^{a}	2^{a}	2^{a}	$3.00±0.57^{a}$
5	1ppm	1^{a}	1^{a}	3^{a}	$1.60±1.15^{a}$

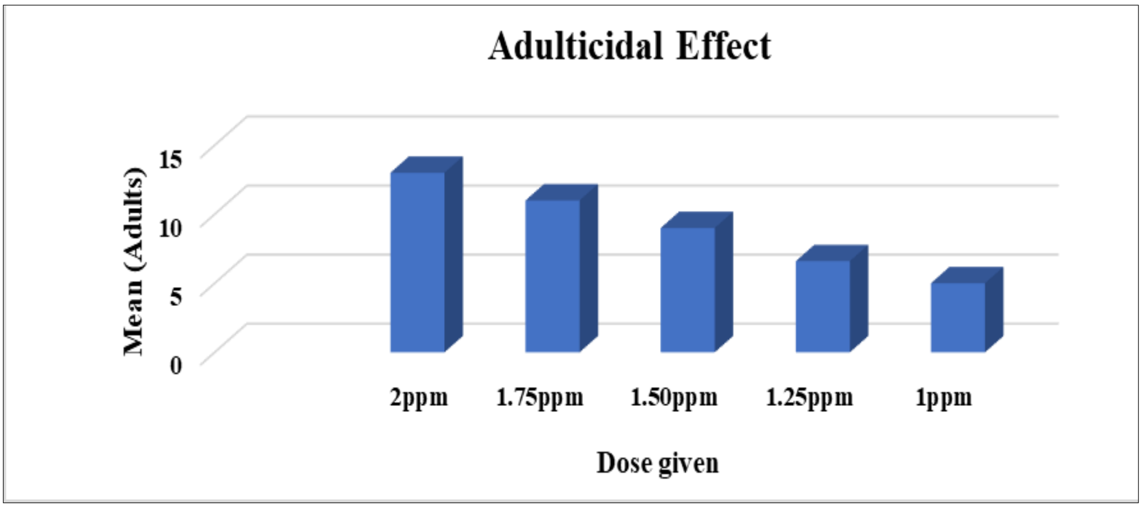
*Statistical analysis (ANOVA) showed significant result with p value = 0.05

*Mean value followed by same letter (a,b,c) in the given table above are not significantly different as per Tukey' Test

*(Mean±S.D.) of three replications

Table 2: Toxicity of aqueous extract against Larvae of *T. castaneum*

S. No.	Dose (ppm)	R1	R2	R3	Mean±SD
1	2ppm	14^{c}	12^{c}	15^{c}	$13.00±1.52^{c}$
2	1.75ppm	12^{bc}	10^{bc}	12^{bc}	$11.00±1.15^{bc}$
3	1.50ppm	10^{b}	9^{b}	10^{b}	$9.00±0.57^{b}$
4	1.25ppm	7^{a}	7^{a}	6^{a}	$6.60±0.57^{a}$
5	1ppm	5^{a}	6^{a}	5^{a}	$5.00±0.57^{a}$



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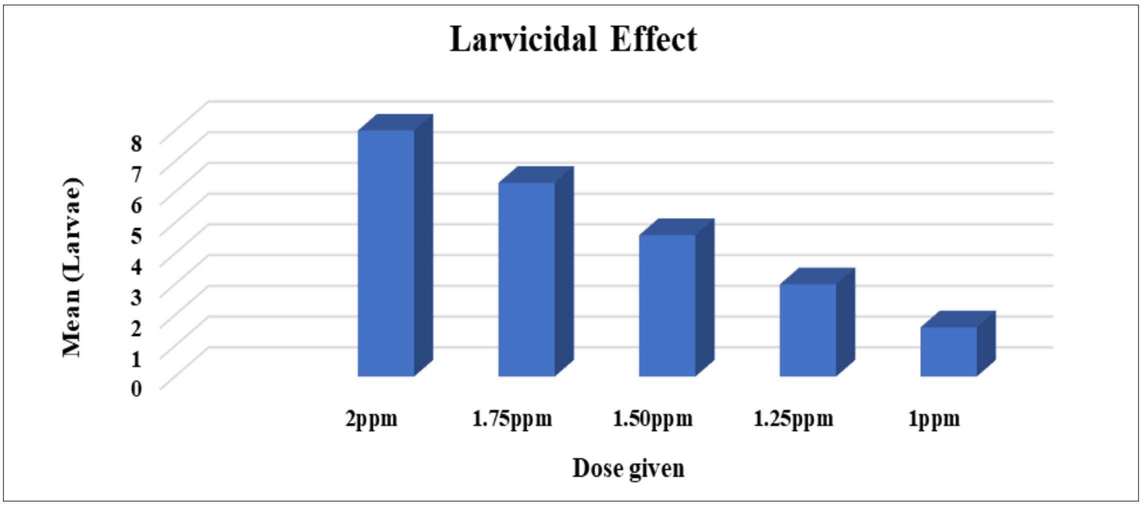
*Mean value followed by same letter (a,b,c) in the given table above are not significantly different as per Tukey' Test
*(Mean±S.D.) of three replications

Fig 1: Toxicity of aqueous extract against Adults of *T. castaneum*



*Statistical analysis (ANOVA) showed significant result with p value = 0.05
*Mean value followed by same letter (a,b,c) in the given table above are not significantly different as per Tukey' Test
*(Mean±S.D.) of three replications

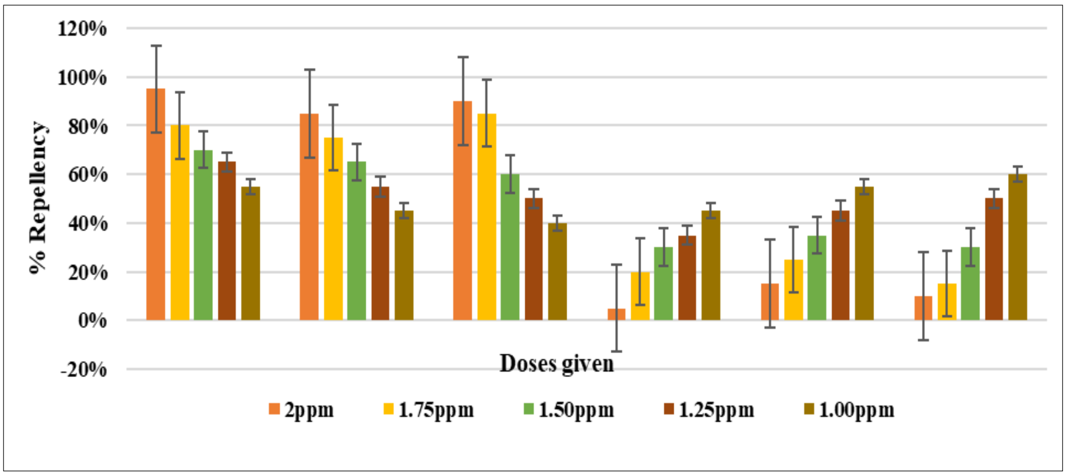
Fig 2: Toxicity of aqueous extract against Larvae of *T. castaneum*

Table 3: Repellency Data of Aqueous extract against adults of *T. castaneum*

S.No.	Dose (ppm)	No. of insects/ trial	No. of adults in untreated area			No. of adults in treated area		
			R1	R2	R3	R1	R2	R3
1	2ppm	20	19	17	18	1	3	2
2	1.75ppm	20	17	16	15	3	4	5
3	1.50ppm	20	14	13	12	6	7	6
4	1.25ppm	20	13	11	10	7	9	10
5	1.00ppm	20	11	9	8	9	11	12

Table 4: Repellency percentage of aqueous extract against adults of *T. castaneum*

Sr. No.	Dose	Untreated area			Treated area		
		R1	R2	R3	R1	R2	R3
1	2ppm	95%	85%	90%	5%	15%	10%
2	1.75ppm	80%	75%	85%	20%	25%	15%
3	1.50ppm	70%	65%	60%	30%	35%	30%
4	1.25ppm	65%	55%	50%	35%	45%	50%
5	1.00ppm	55%	45%	40%	45%	55%	60%

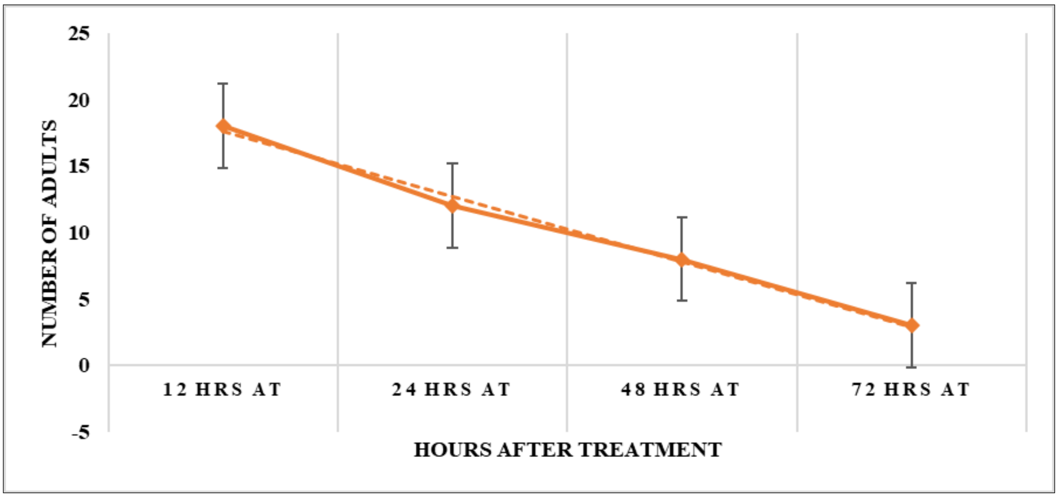


*Statistical analysis (ANOVA) showed significant result with p value = 0.05
*Mean value followed by same letter (a,b,c) in the given table above are not significantly different as per Tukey' Test
*(Mean±S.D.) of three replications

Fig 3: Repellency percentage of aqueous extract against adults of *T. castaneum*

Table 5: Residual toxicity: Mean Mortality of *T. castaneum* by aqueous extract at various Hrs after treatment (AT)

Compound	12 Hrs AT	24 Hrs AT	48 Hrs AT	72 Hrs AT
Curry leaf extract	18	12	8	3
Control	Nil	Nil	Nil	Nil



*Insect no – 20 adults *AT = After Treatment

Fig 4: Residual toxicity: Mean Mortality of *T. castaneum* by aqueous extract at various Hrs After Treatment (AT)

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