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Phytochemical efficiency, fumigant toxicity and repellent activity of certain plant extract against storage rice weevil (*Tribolium castaneum*)

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Abstract An assessment was made about the phytochemical constituents of ethanolic extract of *Cassia auriculata, Moringa oleifera*, and *Plectranthus amboinicus*, as well as fumigant toxicity and repellent activity against the rice weevil, *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae). A variety of phytochemical components were evaluated by the crude extract, including alkaloids, phenols, steroids, reducing sugars, tannins, anthraquinones, terpenoids, flavonoids, and saponins. Using the topical application toxicity method, the adult beetles were exposed to plant extracts for 24, 48, and 72 hours. *Cassia auriculata* expressed the greatest mortality of (63.2±0.8% and LC50 value was 31.62(mg/ml)) in *Tribolium castaneum,* followed by *Plectranthus amboinicus* (55.22±0.8% & LC50 value was 44.66 mg/ml), and *Moringa oleifera* (47.2±0.8% and LC50 value was 63.09 mg/ml) after 72 hours. *Cassia auriculata* was also found to be highly repellent against rice pests with 42.4±0.97 %. It is recommended that phytochemical activity expressed an effective protection of stored grains from *Tribolium castaneum*.

Keywords: *Cassia auriculate*, *Moringa oleifera, Plectranthus amboinicus*, Phytochemical, *Tribolium castaneum*

Introduction

All across the world, people are constantly involved in protecting stored goods from deterioration primarily caused by insects and fungi, which often work together. A large volume of commodities is being kept in storage such as cereal grains, and they represent a vital part of the world food supply. During storage after harvest, grain is typically being kept in the farm or in large storage plants, where it can be infested with a variety of beetles. *Tribolium castaneum* Herbst (Coleoptera Tenebrionidae) is one of the largest and most dangerous pests of stored products, feeding on a wide variety of stored grains (Weston and Rattlingourd, 2000; Mishra *et al.,* 2012a; 2012b). As consumers become less

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tolerant of pesticide residues in their food, grain administrators tend to look only at chemical solutions for controlling stored-grain insects. However, curiosity in non-chemical alternatives to chemical pesticides is growing as grain administrators see a need for managing these pests (Flinn and Hagstrum, 2001). Due to the stringent regulations regarding the safe use of synthetic insecticides on or near food, the choice of pesticides for storage pest control is limited. Furthermore, the continuous use of chemical pesticides for controlling stored grain pests has resulted in serious problems such as insecticide resistance (Mohan *et al.,* 2010). Studies involving novel strategies of pest control like the use of botanical insecticides have been encouraged by the evidence of damage resulting from the uncritical use of synthetic insecticides. The use of phytochemicals with insecticidal properties is one of the most significant locally available, biodegradable and inexpensive methods for controlling stored-grain pests (Mishra *et al.,* 2012b). A major advantage of botanicals is that they can be produced by farmers and small-scale industries and they are probably less expensive (Nikkon *et al.,* 2009).

Using plant-based insecticides to protect stored products is promising mainly due to the possibility of controlling the conditions inside storage units and maximizing the insecticidal effects. (Guzzo *et al.,* 2006). As long as extreme care will be taken to avoid propagating plants from foreign ecosystems, using plants for storage protection is easily maintained, propagated year after year, biodegradable and does not adversely affect the environment (Golob *et al.,* 1999). Numerous plants that are usually observed as safe, however, may contain harmful compounds, which could make them unsafe for humans and animals to consume (Golob *et al.,* 1999; Suthisut *et al.,* 2011). Many medicinal plants are toxic to *Tribolium castaneum* and several locally available species have repellent properties (Sighamony *et al.,* 1984, Obeng-Ofori *et al.,* 1998, Golob *et al.,* 1999, Mareggiani *et al.,* 2000, Nikkon *et al.,* 2009, and Suthisut *et al.,* 2011). There has been shown the evidence that *Ambrosia tenuifolia* Spreng. (Asteraceae), *Baccharis trimera* (Less.) Asteraceae), *Brassica campestris* L. (Brassicaceae), *Jacaranda mimosifolia* D.Don (Bignoniaceae), *Matricaria chamomilla* L. (Asteraceae), *Schinus mole* (L.) var. areira (L.) DC (Anacardeaceae), *Solanum sisymbriifolium* Lam. (Solanaceae), *Tagetes minuta* L. (Asteraceae) and *Viola arvensis* Murray (Violaceae) have insecticidal activity against *Tribolium castaneum*, a coleopteran pest of stored grains (Padín *et al.,* 2000, Tsao *et al.,* 2002, Al-Jabr, 2006, Juan Hikawczuk *et al.,* 2008, Benzi *et al.,* 2009 and Arora *et al*., 2011). Furthermore, these plant species are easily available to local farmers either as decorative plants or medicinal plants, as well as barely being attacked by insects. This study examined the insecticidal potential of ethanolic leaf extracts from *Cassia auriculata*, *Moringa oleifera* and *Plectranthus amboinicus* against the rice weevil, *Tribolium castaneum*

Materials and methods

Plant material collection

The plant materials were collected from different sites in Periyakovilankulam village of Sankarankovil taluk, including *Cassia auriculata, Moringa oleifera* and *Plectranthus amboinicus*. After the leaves were collected, they were shade dried at room temperature for about a month. Powders was prepared by using a pestle and mortar and sieved through 60 mesh size sieves. The powders were stored at room temperature in polythene bags and sealed properly to prevent deterioration of quality. Dr. C. Babu, Associate Professor, Department of Botany, Pioneer Kumaraswamy College, where a voucher specimen has been deposited, identified the plants taxonomically.

Preparation of plant extract

The fresh leaves of *C. auriculata, M. oleifera* and *P. amboinicus* were sun dried for 10 days. The dried plant materials were then powdered with a conventional mixer and stored in a closed container. Using a Soxhlet extractor, 25 g of leaf powder was extracted with 250 ml of solvent for 4 hours at 100°C (Radha *et al.,* 2011). After the crude solvent extracts obtained from the Soxhlet extraction were concentrated using steam distillation, the solvent was evaporated. The evaporated solvent was then decanted into a specimen vial (Kumoro *et al*., 2009). All crude extracts were dried in a laboratory oven at 37°C, weighed to determine the percentage yield of extraction and stored in refrigerator at 4°C.

Phytochemicals screening

The standard qualitative methods were used for phytochemical screening as described by Iqbal Hussain *et al.* (2011). The crude extract was tested using the following tests and reagents, alkaloids with Mayer's and Dragendorff's tests, Phenols with Ferric chloride test, steroids with chloroform and sulphuric acid test, reducing sugars with Fehling's test, tannins with Ferric Chloride test, anthraquinones with Borntrager's test, terpenoids with Salkowski test, flavonoids with ammonia and sulphuric acid, saponins with foam test.

Test for phenols and tannins

An unknown substance was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated that phenols and tannins were present (RNS Yadav and M Agarwala, 2011).

Test for steroid

The crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sideways. The lower layer of chloroform produced a red color, which indicated the presence of steroids. Another test was conducted by mixing the crude extract with 2ml of chloroform. Afterwards, 2ml of each concentrated H_2SO_4 and acetic acid were poured into the mixture. Greenish coloration indicated the presence of steroids (RNS Yadav and M Agarwala, 2011).

Test for glycosides

The crude extract was mixed with 2ml of chloroform. Concentrated H_2SO_4 was then added carefully and gently stirred. A reddish brown colour indicated the presence of the glycone portion of the glycoside (RNS Yadav and M Agarwala, 2011).

Test for terpenoids

A crude extract was dissolved in 2ml of chloroform and evaporated to dryness. The combined mixture was added to 2ml of concentrated H_2SO_4 for about 2 minutes, and a grayish colour indicated the presence of terpenoids (RNS Yadav and M Agarwala, 2011).

Test for saponins

The crude extract was combined with 5ml of distilled water and shaken vigorously to create stable foam, which indicates the presence of saponins in the extract (RNS Yadav and M Agarwala, 2011).

Test for flavonoids-Shinoda test

The crude extract was mixed with a few fragments of magnesium ribbon and concentrated HCl was added dropwise. A pink scarlet color developed after few minutes, indicating the presence of flavonoids (RNS Yadav and M Agarwala, 2011).

Test for alkaloids

The crude extract was mixed with 2ml of 1% HCl and gently heated. Mayer's and Wagner's reagents were added to the mixture. Turbidity of the precipitate resulting from the mixture was evidence for alkaloids (RNS Yadav and M Agarwala, 2011).

Test for proteins-Millon's test

When crude extract was mixed with 2ml of Millon's reagent, a white precipitate formed that turned red upon gentle heating, indicating that protein was present (RNS Yadav and M Agarwala, 2011).

Pest collection and rearing

Adult of *T. castaneum* insects were obtained from infested rice, identified, isolated and raised on rice with 5.0% brewer's yeast under continuous dark conditions at 27±1 °C and 65±5% relative humidity (RH). The experiments were conducted by the PG and Research Department of Zoology, PMT College, Sankarankovil. The adults used were 7–14 days old and were of mixed sex. For the experiments, rice was stored at –24 °C for at least 2 days and then kept in incubators set at 27±1 °C and 55±5% RH for a week to obtain moisture content as related to the environmental RH.

Repellency tests

A method of area partiality, used to test the repellent effects of the plant extract and some of the plant's individual ingredients against *T. castaneum*, was used to test the repellent properties (Tapondjou *et al*., 2005). A volume of 1 mL of ethanol plant extract solution was applied uniformly to a half-filter paper disk in all cases to obtain the desired plant extract concentration per unit area of 10, 20, 30, 40, and 50 mg/ml, using ethanol as a solvent. Half of the filter paper was treated with an equal volume of ethanol as a control. The test areas consisted of 9 cm Whatman no. 1 filter paper cut in half (31.8 cm^2). The treated and control half disks were air dried for 10 minutes. The treated and untreated halves were reattached using adhesive tape and placed in 90 mm glass Petri dishes. Thirty adult *T. castaneum* were released at the center of each filter paper disk. Dishes were placed in darkness at 22-2 oC and 75± 10% RH for 60, 120, 180, 240, 300, and 360 seconds. The number of *T. castaneum* on the treated and untreated halves of the experimental paper halves was recorded. Based on an exposure time, the percent repellency (PR) was calculated as follows: PR = [(Nc -Nt)/(Nc-Nt)] x100, where Nc and Nt were the number of insects on the untreated (control) and treated areas, respectively. Five replicates were used for each tested concentration.

Fumigant toxicity

The fumigant study was conducted at 26 ± 1 °C and 65 ± 5% RH using newly developed adults (1–15 days old). A fumigant method based on three-plant extracts was tested against adult of *T. castaneum*. Glass jars (1 L) were made into fumigation chambers (replicas) and pieces of filter paper were joined to the screw cap. Plant extracts were added to each piece of filter paper in increments of 10, 20, 30, and 40 mg/ml. Each replicate jar containing 30 insects was tested five times. Filter paper pieces were treated with ethanol alone as a control. Control insects were kept under the same conditions with ethanol. Insect mortality percentage was observed after 24, 48, and 72 hours of treatment and the lethal concentration causing 50% mortality (LC_{50}) in mg/ml from log-concentration mortality regression lines was calculated. Insects were considered dead if there were no movements of their legs or antennae (Abbott, 1925; Schultz, 1989 and Muhammad, 2008).

Statistical analysis

The data were analyzed using the one-way analysis of variance (ANOVA) and the least significant difference (LSD) multiple range test to determine significant ($P < 0.05$) differences among concentrations (STSC Inc., Rockville, M.D). Finny (1971) conducted probit analysis to estimate lethal concentrations (LC50) and significant differences in LC values were considered when their respective 95% fiducial limits did not overlap.

Results

Phytochemical screening

A qualitative analysis of the phytochemicals in the leaf extracts of *C. auriculata*, *M. oleifera* and *P. amboinicus* is shown in Table 1.

Repellent activity

All three plant extracts tested were highly repellent to *T. castaneum* and the concentration-response analyses were significant (Table 2 and Figure 1). It was discovered that the repellent activity of the plant extracted from *C. auriculata* was significantly influenced by the concentration applied and interestingly, was also increased when insects were exposed for a longer period of time. When the extracts were applied at concentrations of 10, 20, 30, 40 and 50 mg/ml for 6 hours, the PR values ranged from 27.2% to 42.4%.

Table 1. Phytochemical analysis of plant extracts

Phytochemical Testes	*Moringa oleifera*	*Plectranthusamboinicus*	*Cassia auriculata*
Phenols and Tannins	+	++	+++
Steroids	+	+	+++
Glycosides	-	-	+
Terpenoids	+	++	+++
Saponins	-	+	+++
Flavonoids	-	+	++
Alkaloids	+	++	+++
Protein	-	+	++

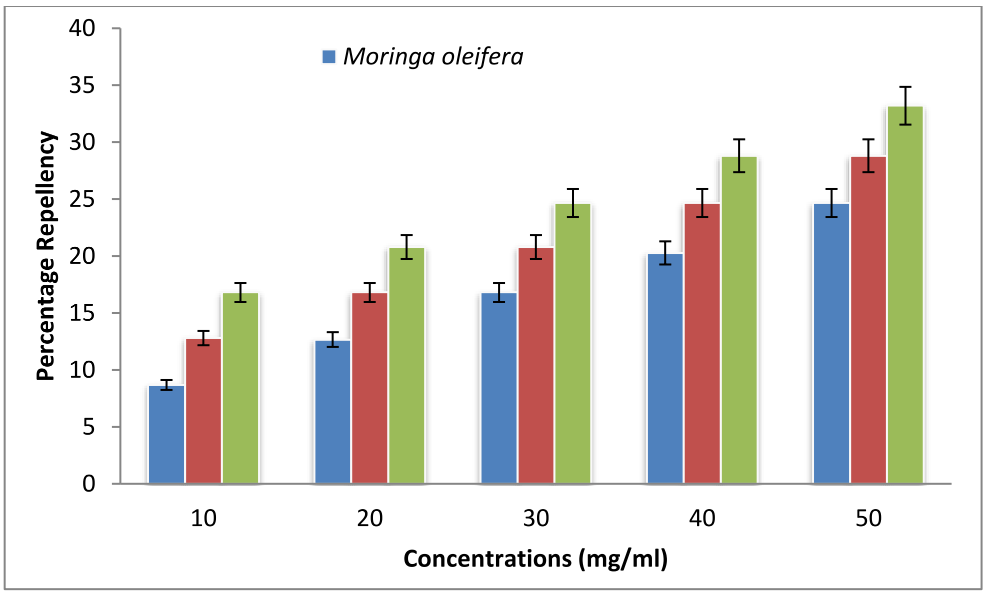

Figure 1. Percentage Repellency of *Moringa oleifera, Plectranthus amboinicus* and *Cassia auriculata* against *Tribolium castaneum*

Fumigant toxicity test

The three plant extracts showed a strong fumigant activity against *T. castaneum* and the time required to cause 50% mortality (LC50) decreased with increasing concentrations of the oils (Tables 3, 4 and Figures 2, 3). Using plant extracts from leaves, insects were fumigated at concentrations of 10, 20, 30, 40, and 50 mg/ml and exposure times of 24, 48, and 72 hours.

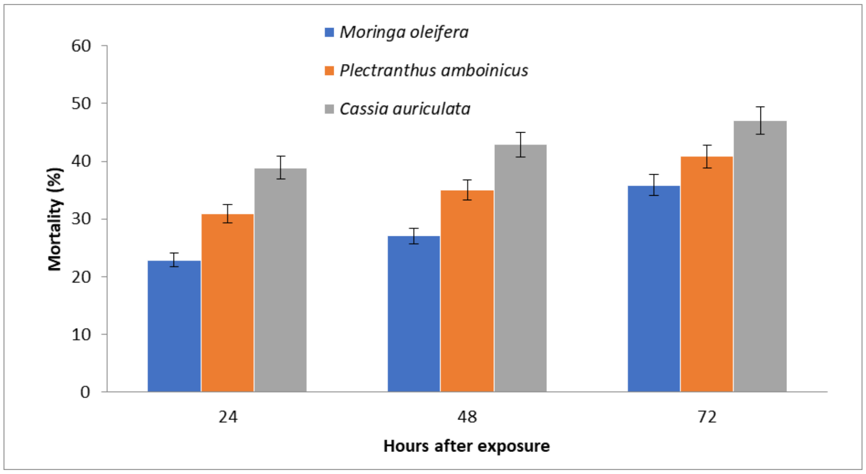

Figure 2. Mortality of Triboliumcastaneum when treated with *M. oleifera*, P. *amboinicus* and *C. auriculata*

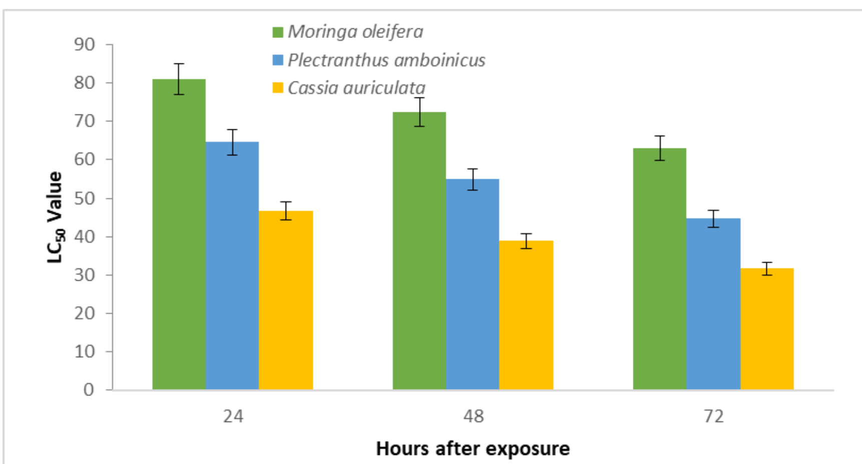

Figure 3. LC_{50} Value of three plant extracts against rice weevil

Table 2. Percentage Repellency of *M. oleifera*, P. *amboinicus* and *C. auriculata* against *T. castaneum* (%)

Plant Material	concentration	60	120	180	240	300	360	Mean	P value	Significance
Moringa oleifera	10	-1.6±1.6	4.8±0.8	7.2±0.8	9.6±0.97	14.4±0.97	17.6±0.97	8.67	0.000388	**
	20	3.2±0.8	6.4±0.97	11.2±0.8	13.6±0.97	18.4±0.97	23.2±0.8	12.67		
	30	6.4±0.97	11.2±0.8	14.4±0.97	19.2±0.8	22.4±0.97	27.2±0.8	16.80		
	40	9.6±0.97	15.2±0.8	17.6±0.97	21.6±0.97	27.2±0.8	30.4±0.97	20.27		
	50	14.4±0.97	18.4±0.97	23.2±0.8	26.4±0.97	31.2±0.8	34.4±0.97	24.67		
Plectranthusamboinicus	10	2.4±0.97	7.2±0.8	10.4±0.97	15.2±0.8	18.4±0.97	23.2±0.8	12.80	0.000321	**
	20	7.2±0.8	10.4±0.97	15.2±0.8	18.4±0.97	23.2±0.8	26.4±0.97	16.80		
	30	10.4±0.97	15.2±0.8	18.4±0.97	23.2±0.8	26.4±0.97	31.2±0.8	20.80		
	40	14.4±0.97	18.4±0.97	23.2±0.8	26.4±0.97	31.2±0.8	34.4±0.97	24.67		
	50	18.4±0.97	23.2±0.97	26.4±0.97	31.2±0.8	34.4±0.97	39.2±0.8	28.80		
Cassia auriculata	10	6.4±0.97	11.2±0.8	14.4±0.97	19.2±0.8	22.4±0.97	27.2±0.8	16.80	0.000592	**
	20	11.2±0.8	14.4±0.97	19.2±0.8	22.4±0.97	27.2±0.8	30.4±0.97	20.80		
	30	14.4±0.97	18.4±0.97	23.2±0.8	26.4±0.97	31.2±0.8	34.4±0.97	24.67		
	40	19.2±0.8	22.4±0.97	27.2±0.8	30.4±0.97	35.2±0.8	38.4±0.97	28.80		
	50	22.4±0.97	29.6±0.97	31.2±0.8	34.4±0.97	39.2±0.8	42.4±0.97	33.20		

** Highly significant

Table 3. Mortality of *T. castaneum* when treated with *M. oleifera*, P. *amboinicus* and *C. auriculata* against

Plant Material	Concentration (mg/ml)								
	Hours	10	20	30	40	50	Mean	P value	Significance
Moringa oleifera	24	7.2±0.8	15.2±0.8	23.2±0.8	30.4±0.98	38.4±0.98	22.88	0.005	**
	48	11.2±0.8	18.4±0.98	27.2±0.8	35.2±0.8	43.2±0.8	27.04	0.007	**
	72	14.4±0.98	23.2±0.8	31.2±0.8	39.2±0.8	47.2±0.8	35.84	0.08	**
Plectranthusamboinicus	24	14.4±0.98	22.4±0.98	32±0.0	38.4±0.98	47.2±0.8	30.88	0.007	**
	48	19.2±0.8	26.4±0.98	35.2±0.8	43.2±0.8	51.2±0.8	35.04	0.008	**
	72	22.4±0.98	32±0	39.2±0.8	48±0.0	55.2±0.8	40.8	0.08	**
Cassia auriculata	24	22.4±0.98	31.2±0.8	39.2±0.8	47.2±0.8	54.4±0.98	38.88	0.06	**
	48	27.2±0.8	34.4±0.98	43.2±0.8	50.4±0.98	59.2±0.8	42.88	0.005	**
	72	30.4±0.98	40±0.0	46.4±0.98	55.2±0.8	63.2±0.8	47.04	0.004	**

**highly significan

Table 4. LC_{50} Values of three plant extracts against rice weevil *T. castaneum*

Plant Materials	Hours	Intercept	X Variable	LC_{50}(mg/ml)
M. oleifera	24	1.847165113	1.647672807	81.09
	48	2.214543912	1.496964137	72.44
	72	2.450328659	1.418010975	63.09
P. amboinicus	24	2.467745074	1.405709825	64.56
	48	2.773553406	1.281537038	54.95
	72	2.925411207	1.261861175	44.66
C. auriculata	24	2.956835523	1.22695296	46.77
	48	3.157273326	1.164489661	38.90
	72	3.261694635	1.169842463	31.62

Discussion

The ethanolic extract of *C. auriculata* contained the highest amount of phenolics, steroids, glycosides, tannins, terpenoids, saponins, alkaloids, proteins and flavonoids. *P. amboinicus* leaf extracts showed a moderate number of phytochemicals, such as tannins, terpenoids, saponins, alkaloids, proteins, and flavonoids. *M. oleifera* extract contained the least amount of phytochemicals, such as tannins, terpenoids, flavonoids, saponins, and proteins. *C. auriculata* contained more phytochemicals than other plant extracts. Some secondary metabolites, such as terpenoids and alkaloids, have been reported as possible insecticides (Schultz, 1989) that may be concerned an effective alternative for controlling insect pests. Secondary metabolites (terpenes, alkaloids, steroids, flavanoids, phenols, tannins, sugars, gums, etc.) are essential for protecting plants from microbial pathogens and invertebrate pests. (Gottlieb, 1990 and Wink and Schimmer, 1999). Compounds with alkaloids have insecticidal properties at low concentrations. Their mode of action varies, but many affect the nervous system's acetylcholine receptors or the sodium channels in nerve membranes. Alkaloids can be found in huge quantities in many members of the Fabaceae and Solanaceae families, all of which have long been used as traditional insect repellents (Secoy and Smith, 1983; Johnson, 1998). *C. auriculata* belongs to Fabaceae family.

Phenolic compounds are characterized by the presence of a hydroxyl group attached to a benzene ring or to another complex aromatic ring structure. According to various studies, plant phenolics are one of the most effective defenses against insects (Berbehenn and Martin, 1994; Berbehenn *et al*., 1996; Henn, 1997). Terpenes are the largest group of natural products from plants, including oils, flavors, fragrances and pigments which are lipid-soluble. These hydrophobic compounds are generally stored in resin ducts, oil cells or glandular trichomes in plants. (Wink and Schimmer, 1999). Monoterpene esters cause paralysis and death in animals, which led to the development of some of the most effective commercial pesticides (Raffa and Priester, 1985; Gershenzon and Croteau, 1994).

Both plants extracts were moderately effective in repelling in *T. castaneum*. *M. oleifera* extract had the lowest repellent activity and ranged from 17.6 to 23.2. Our findings suggested that *T. castaneum* adults were repelled even by very low concentrations of plant extract. The repellent properties of *C. auriculata* were better than those of *P. amboinicus* and *M. oleifera*. A plant extract from the Fabaceae family has shown to have powerful repellent properties. This is because the Fabaceae family contains a high number of alkaloid compounds. (Secoy and Smith, 1983; Johnson, 1998). *C. auriculata* belongs to the Fabaceae family of plants and contains high quantities of alkaloids. These extracts, exhibit better repellency than plants with a lower concentration of alkaloids.

Mortality activity of *M. oleifera* was observed to be 22.88, 27.04, and 35.84 in 24, 48, and 72 hours, respectively. *P. amboinicus* plant extract, is observed the mortality percentage was 30.88 (24hr), 35.04 (48hr), and 40.08 (72hr). *C. auriculata* showed a mean value of mortality were 38.88, 42.88 and 47.04 % respectively in 24, 48, and 72 hours. *C. auriculata* showed more toxic than *P. amboinicus* and *M. oleifera*. An example of a well-studied compound found in Chrysanthemum species leaves. As a result of the toxin causing paralysis and mortality, pesticides were developed to be the most successful commercially (Raffa and Priester, 1985; Gershenzon and Croteau, 1994). *Moringa oleifera* showed LC_{50} values of 81.09 (mg/ml), 72.44 (mg/ml), and 63.09 (mg/ml) in 24, 48, and 72 hours, respectively. *C. auriculata* showed LC_{50} values of 46.77 (mg/ml), 38.90(mg/ml), and 31.62(mg/ml) while *P. amboinicus* showed 64.56(mg/ml), 54.95(mg/ml), and 44.66(mg/ml). Because Cassia auriculata contains a greater quantity of terpenoids, it was more toxic and had higher mortality rate than the other extracts. Studying the effectiveness of botanical derivatives as insecticides will benefit agricultural sectors in developing countries, since these substances are not only low-cost but also have a comparatively low environmental impact (Upadhyay and Jaiswal, 2007, Pirasanna Pandi *et al*., 2018).

Based on the results of the present investigation, it is possible to develop the use of botanical compounds as insecticides commercially. *C. auriculata* leaves powder extract displayed higher levels of toxicity and repellency against *T. castaneum*. Traditional plant products have been found to be highly effective at controlling the rice beetle *T. castaneum* in order to reduce the severity of damage caused by insects. It is a simple and effective method for storing rice with *C. auriculata* extracts that consists of easy adaptability as well as cost effectiveness. It should be accepted by farmers in the near future because of these advantages. However, further research is needed to isolate and identify the active principal component of the product, as well as to evaluate its cost-benefit ratio and its ability to control insect infestations in rice stores.

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