



INSECTICIDAL AND ANTIFEEDANT EFFECT OF MEDICINAL PLANT EXTRACTS AGAINST RED FLOUR BEETLE *TRIBOLIUM CASTANEUM*

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Article Info:

Research Article
Received

30.04.2024

Reviewed

10.06.2024

Accepted

01.07.2024

Abstract: *Tribolium castaneum* commonly called Red flour beetle is a widely distributed stored grain pest. The present study was designed to evaluate the effect of five medicinal plant extracts against *T. castaneum* under laboratory conditions. Five doses in five replications for each of the plant powder were prepared by mixing them with flour inside plastic jars. Ten pairs of adult beetles were placed inside each jar and mortality was recorded. Feeding deterrent index (FDI) was calculated. At the 4th day (96 hours after treatment) maximum mortality is seen in case *Murraya koenigii* followed by *Mentha spicata*, *Justicia adhatoda*, *Centella asiatica*, *Hygrophila polysperma*. The ANOVA result revealed that the ethanol extract of *Murraya koenigii* is the most toxic against the pest in comparison to other plant extracts.

Keywords: ANOVA, Ethanol extract, Feeding deterrent index, Mortality rate, *Tribolium*.

Cite this article as: Dutta A. and Sarkar S. (2024). Insecticidal and Antifeedant effect of medicinal plant extracts against Red flour beetle *Tribolium castaneum*. *International Journal of Biological Innovations*. 6(2): 83-90. <https://doi.org/10.46505/IJBI.2024.6202>

INTRODUCTION

The protection of stored grain and seeds against insect pests has been a major problem from the development of agriculture. The indiscriminate use of chemical pesticide over a long period has not only been proved to be harmful to soil microflora, animals and human life, but also contributed to a number of side effects (Prakash and Verma, 2014; Ashok, 2019; Verma and Prakash, 2018). The development of resistance by the insects/weeds/pests resurgence and outbreak of new pests, toxicity to non-target organism, presence of non permissible level of pesticide

residues on seeds, vegetables, fruits, border alteration in dynamics of pest species population, cumulatively causing poor soil fertility and hazardous effects on environment endangering the sustainability of ecosystem (Rani *et al.*, 2024). The pesticides and other human activities cause pollution that is harmful for overall biodiversity (Prakash and Verma, 2022; Singh *et al.*, 2023).

Plant products have been successfully used as insecticides, insect repellents and insect antifeedants. Higher plants are with a rich source of novel natural substance that can be used to



develop environmental safe methods for insect control (Arnason *et al.*, 1989). The deleterious effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant growth inhibitor, suppression of reproductive behaviour and reduction of fecundity and fertility. Besides, many plants have ethno-medicinal values (Rao, 2019; Sharma and Pareek, 2021).

Insect pests cause damage to stored grain and processed products by reducing dry weight and nutritional value. *Tribolium*, the flour beetle is a serious stored product pest, which attacks several crops, including wheat and model organism for development and functional genomics. The flour beetle is the most widespread and destructive major insect pest of stored cereals throughout the world. The flour beetles consume wheat, other grains and are adapted to survive in very dry environment, and can withstand even higher amounts of radiation than cockroaches. Female red flour beetles are polyandrous in mating behavior. They cannot feed on whole undamaged grain and occur in dust, fines, and dockage. The beetles do cause damage by feeding but probably cause more problems by contaminating the grain. The manifest consequences are the gradual depletion of food quality over storage time resulting to poor products. Hence, the post-harvest loss (PHL) due to the pest attack and other factors in developing countries posed threats to food security. This can be achieved through qualitative research and development strategy on plant based pesticides, which have been found to act concertedly on both behavioural activities and physiological processes of insect.

To minimize the use of synthetic pesticides and to avoid pollution of the environment, natural antifeedant, deterrent and repellent substances have been searched for pest control during recent times. However, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and environment friendly. Considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides. Botanical insecticides are often effective alternatives to organophosphates or other neurotoxins for pest control

due to multiple modes of action. These include toxicity, antifeedant and anti-oviposition effects. Natural products containing secondary plant compounds such as terpenes, steroids, alkaloids, phenolics and glycosides affect insect behavior and are toxic in some cases (Duke, 1990; Mordue *et al.*, 1998).

In the context of agricultural pest management, botanical insecticides can play a much greater role in the production and postharvest protection of food in developing countries who have the best supplies of the natural resource and have the most to gain from the development and local use of simple plant extracts for crop protection (Koul and Dhaliwal, 2001; Isman, 2006). In many countries, plant tissues or crude products of the plants, such as aqueous or organic solvent extracts, are used directly as protectants of stored products. These practices are labour intensive, but are often economically and ecologically sound, and do not require sophisticated technology (Rahman and Talukder, 2006).

Farmers and researchers often claim successful use of material of plant origin in insect pest control including spices and powders of plant parts (Akinneye *et al.*, 2006). Previous research indicated that some plant powders, oils and extracts have strong effect on stored grain insects such as toxicity and the inhibition of reproduction (Emeasor *et al.*, 2005; Nadra, 2006). The simplest way to apply plants to a stock of seeds is harvesting the plant and adding it to the seeds. The modes of action of powders vary, but with low to moderate dosages, the effect is always repellent or toxic, never mechanical. For the last few years the influence of different plant dried materials (added as powder to the products the pests feed on) on the cereal and flour pests have been widely explored (Blazejewska and Wyrostkiewicz, 1998). The use of spices is also less costly, easily available, safer and don't do any hazard using in the stores (Aslam *et al.*, 2002; Mahdi and Rahman, 2008).

Considering these facts, the present investigation was aimed to evaluate the antifeedant activity of dried powdered material as well as ethanol extract of leaves of Basak (*Justicia adhatoda*:

Acanthaceae), Pudina (*Mentha spicata*: Mentheae), Thankuni (*Centella asiatica*: Apiaceae), Kulekhara (*Hygrophila polysperma*: Acanthaceae) and Curry (*Murraya koenigii*: Rutaceae) of the major storage insect pest of worldwide distribution.

MATERIALS AND METHOD

1. Insect culture:

The experiment was conducted in the Entomology laboratory in the Department of Zoology, Serampore College, Hooghly, India. The wheat containing larva and adult *Tribolium* sp. was collected from local market, after that wheat was kept in a dark place at room temperature for increasing the number for two month. The homogeneous population of *Tribolium* sp. in wheat was maintained at 25 ± 2 °C and 70 ± 5 % R.H. under the laboratory condition.

2. Preparation of dried plant materials:

Fresh leaves of Basak, Kulekhanra, Pudina, Thankuni and Curry were collected from trees. These leaves were washed, air dried and ground to a fine powder using a domestic electric grinder (Fernando and Karunaratne, 2012). Dried powdered plant-material obtained in this manner was used for all experiments.

3. Extraction by Soxhlet apparatus:

For the extraction, crude extracts of the dried plants leaves of Basak, Kulekhanra, Pudina, Thankuni and Curry were used. These leaves were ground in an electric grinder to obtain a powder. Maceration was performed in solvents of different polarity (Ethanol) for 72h with the aim of extracting different plant components. The extraction of each plant sample was done in about 12h. Soxhlet Extraction Apparatus was used to extract plant leaves compounds by dipping 50g of powder in 250ml ethanol according to the procedure described (Valladares *et al.*, 1997). The plant leaves were done by placing flasks in the Soxhlet Apparatus. These leaves were poured into a filter tube made from filter paper on one end of the cap. A flask which contained 500 ml of ethanol was placed under this glass tube 3 times for 24h per batch. After 3 days, the solvent was evaporated by heat lamp. The extracts were stored at 4°C prior to application.

4. Rearing and culture of insect:

Population of *T. castaneum* was cultured in a controlled environmental room at 29°C and 75% R.H under continued dark photoperiod. The food media was broken wheat grain (Padin *et al.*, 2013). They were obtained from the laboratory stock culture. The *S. oryzae* lesser grain borer was reared on uninfected whole rice (Ashouri and Shayesteh, 2010). One hundred *T. castaneum* adult of mixed sex were put in 500ml glass jars containing 200g of wheat grains (Daniel *et al.*, 2013). The jars were covered with muslin cloth held in place with rubber band for ventilation. The jars were kept in the laboratory maintaining temperature at 29 ± 2 °C and relative humidity at 70 ± 5 % respectively, for two months to multiply insect number. To ensure continuous supply of adequate adults, the rearing procedure was repeated with different batches of insect. After 48h, the adults and subsequently these adults were used for the experiments.

5. Contact/ Feeding Toxicity Test with respect to percentage of adult mortality:

Dried powdered materials and ethanol extracts of four plants weighing 3.0g, were mixed with 50g of white wheat in separate plastic containers (height 8 cm, diameter 5 cm) using a glass rod. Ten adult beetles were then introduced into each container and the mouth of the container was covered with a muslin cloth. In the control, 20 adult insects were introduced into 50g of untreated wheat. The number of dead adult weevils in each container was recorded after 24, 48, 72, 96 hours after their introduction. This experiment was replicated 5 times with 5 replicas of each experimental set. The mortality was assessed by means of direct observation, and when no leg or antennal movements were observed, the insects were considered dead. Mortality was calculated and corrected as per Abbott's formula (Abbott, 1925).

Mortality (%) = [(percent treated mortality – percent control mortality) / (100 – percent control mortality)] x 100

6. Experimental Set-up:

The antifeedant parameter feeding deterrent index (FDI) was calculated. Following formula was used for calculating the nutritional indices:

FDI = $[1 - T/C] \times 100$

Where C= consumption of control disc, T = consumption of treated discs. The classes of repellence (% repellence rate) were categorized; according to

The classes of Repellence (% repellence rate) were categorized; according to Mc Donald *et al.* (1970). ANOVA was done to test the differences among various means with JMP SAS and IBM SPSS 20 package.

RESULTS AND DISCUSSION
This experiment was conducted in order to determine the insecticidal activity of the plant powder used on *Tribolium castaneum*. Authors recorded a considerable difference in insect mortality feeding deterrence in all the cases, which were shown with different plant powders as well as ethanol extract of plants. All the results are presented from tables 1 to 4 and graph 1.

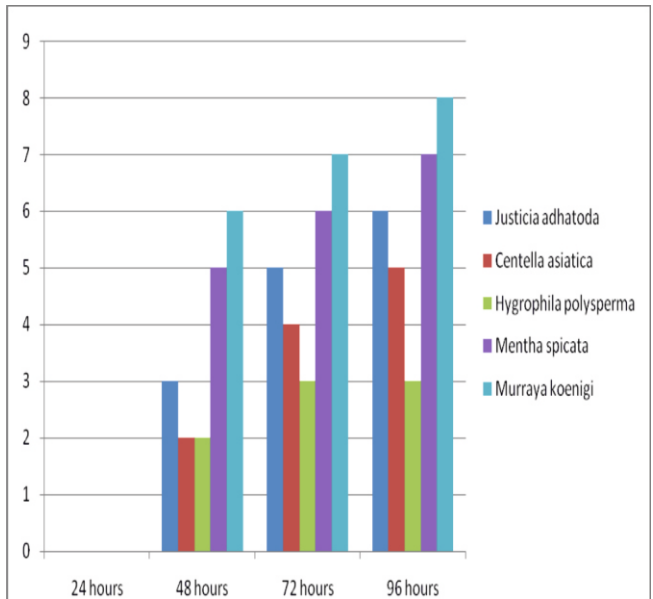
Table 1: Feeding toxicity of ethanol extract of plant material at different time interval.

Treatment of plant extract		Number of adult died in treatment and control																								
		<i>Justicia adhatoda</i>					<i>Centella asiatica</i>					<i>Hygrophila polysperma</i>					<i>Mentha spicata</i>					<i>Murraya koenigii</i>				
Hrs	Control	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
24	0		0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
48	0	0	0	1	1	2	0	0	0	1	1	0	0	0	0	1	1	1	1	2	1	1	1	1	2	2
72	0	0	1	1	1	2	0	0	1	1	2	0	0	0	0	1	2	1	1	2	1	3	1	1	1	3
96	0	2	1	1	2	2	1	2	1	1	1	1	0	0	1	0	2	2	2	1	2	4	1	1	1	5

Table 2: Efficiency of ethanol extract of plant on mortality.

Treatment of plant extract (300 mg)	% of Adult mortality exposed periods (hrs. after treatment)			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.
<i>Justicia adhatoda</i>	01	04	05	08
<i>Centella asiatica</i>	00	02	04	06
<i>Hygrophila polysperma</i>	00	01	01	02
<i>Mentha spicata</i>	00	05	06	09
<i>Murraya koenigii</i>	02	08	10	12

The contact/ feeding toxicity of different dried plant powder and extract having same dose on *Tribolium castaneum* at different time intervals are shown in Table 1. The efficiency of the plant powder and ethanol extract of plants on mortality of *Tribolium castaneum* is shown in Table 2 for the different time periods. According to the observations, no adult mortality was seen after 24 hours treatment in all cases. Even after 48 hours of treatment adult mortality is seen in case of *Murraya koenigii*. It is seen that in all the four cases mortality gradually increased with the time of exposure (Graph 1). At the 4th day (96 hours after treatment) maximum mortality is seen in case *Murraya koenigii* followed by *Mentha spicata*, *Justicia adhatoda*, *Centella asiatica*, *Hygrophila polysperma*.



Graph 1: Percentage of adult mortality.

The analyzed result of the present study indicates that same dose of powder and ethanol extract of *Murraya koenigii* shows maximum contact/feeding toxicity than that of other species of plants. It is also observed that toxicity effect depends on active biochemicals present in different plants and also time dependents one. All the ethanol extract inhibited the feeding

activity on *Tribolium castaneum* (Table 3), which has pesticide resistance property (Zettler and Cuperus, 1990). The antifeedant activities of ethanol extract of the different plant were significantly different. The maximum feeding deterrent effect was shown in case of *Murraya koenigii*.

Table 3: Calculation of FDI for each ethanol extract of plant material.

Treatment (300 mg) (Ethanol extract)	Wt. (mg) of the live weevil	on 4th day No. of the live weevil	Original Wt. (mg) of weevil on 4th day	Original no. of weevil	Consumption (mg) in control after 4th day	Consumption (mg) in treated after 4th day	FDI (%)
<i>Justicia adhatoda</i>	1550	05	1010	10	800	370	53.75
<i>Centella asiatica</i>	1820	06	1020	10	800	540	32.50
<i>Hygrophila polysperma</i>	1470	09	1050	10	800	610	23.75
<i>Mentha spicata</i>	1390	03	1010	10	800	150	81.25
<i>Murraya koenigii</i>	1190	02	1065	10	800	80	90

In the present study the increase or decrease deterrence with time may be explained on the basis of the physical and chemical properties of the active ingredients present in the plant powder (Koenigiin, Girinimbin, Koenine, Koenidine, Iso-mahanimbin, koenimbine). Triterpenoid alkaloids, Cyclomahanimbine, Murrayastine, Murrayaline, Pyrafoline Carbazole alkaloids and many other chemicals have been isolated from *Murraya koenigii* leaves. Ashouri and Shayesteh (2010) stated that with low molecular weight and high volatility decrease rapidly over time. The study also confirms the capability of the plant powder to keep away insects by way of their repellent or feeding deterrent activity.

Powders of various plant species with insecticidal activity have been used previously by several researchers in laboratory trials for the control of stored product pests including *Tribolium castaneum* (Abida *et al.*, 2010). Large variation in the sensitivity of stored grain pests to fumigation toxicity of volatiles of many plants

have also been reported by several workers (Schmidt *et al.*, 1991; Shaaya *et al.*, 1997; Lee *et al.*, 2001; Tripathi *et al.*, 2002).

The insecticidal activity of botanical products may possibly be dependent on different factors such as the presence of bioactive chemicals with diverse activities. The powdered seeds of *Murraya koenigii* tested may act as a fumigant and a stomach poison. Also the powder may act as a physical barrier blocking the spiracles of the insects, thus impairing respiration leading to their death (Law-Ogbomo and Enobakhare, 2007; Mulungu *et al.*, 2007). Table 4 shows the ANOVA tested values for the different plants extracts on *Tribolium castenum*. The F values of *T. castenum* ranged from 16.80 for *Mentha spicata*, and 3.11 for *Murraya koenigii* but the F values for *Justicia adhatoda*, *Centella asiatica*, *Hygrophila polysperma* were 8.0, 10.87, 3.30 respectively. The most insecticidal effect shown by the plant was *Murraya koenigii* and the least toxic *Mentha spicata*.

Table 4: ANOVA result of treatments on adult mortality.

Treatments	% of Adult mortality exposed periods (hrs. after treatments)				ANOVA
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	
<i>Justicia adhatoda</i>	01 ± 0.57	04 ± 0.57	05 ± 0.57	08 ± 0.57	F- 8.0 p- 0.031
<i>Centella asiatica</i>	00 ± 00	02 ± 0.57	04 ± 0.57	07 ± 0.57	F- 10.87 p- 0.038
<i>Hygrophila polysperma</i>	00 ± 00	01 ± 0.57	01 ± 0.57	02 ± 0.57	F-3.30 p- 0.078
<i>Mentha spicata</i>	00 ± 00	05 ± 0.55	06 ± 0.57	08 ± 0.51	F- 16.80 p- 0.002
<i>Murraya koenigi</i>	02 ± 0.57	08 ± 0.57	10 ± 0.57	12 ± 0.61	F- 3.11 p- 0.267
No mortality was observed in the control; Values are mean ± SEM of 5 replicates; p- 0.05					

CONCLUSION

The findings of the present investigation indicate that botanical derivatives might be useful as store insect control agents for commercial use. All tested plants powder was effective to some degree in reducing the number of *Tribolium castaneum*. The extremely high deterrent activity observed in the present study shows that insecticidal properties of seeds of *Murraya koenigii* could be a source of some biologically active volatile compound that is potentially an efficient insecticide. Consequently, the possibility of utilizing this natural plant products to control stored grain insect pests is worthy for further investigations. Moreover, farmers who are aware of the importance of non- chemical based eco-friendly control methods would certainly prefer to use such ethno-botanical products as stored product protectants. The leaf powdered as well as extract have resulted in high mortality, delay in development and subsequent significant population reduction. Thus, it could be employed as alternatives for chemical pesticides. A study to improve the effectiveness of botanical derivatives as insecticides will benefit the agricultural sectors of developing countries, as the substances are not only of low cost, but also have less environmental impact in terms of insecticidal hazard. The use of such insecticide is

safe, environmental friendly, cheap and versatile. More research can be helpful in developing easy and effective application methodologies for the pest management in domestic and small-scale-field storage as well as large-scale-field storage houses.

ACKNOWLEDGEMENTS

Authors extend their gratitude to Principals of respective colleges for providing laboratory facilities and necessary helps whenever required. Authors are also thankful to Mr Vicky Shaw for his kind help in statistical calculations.

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