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Evaluation of Insecticidal and Repellent Effects of Two Plant Extracts, Acacia and Neem Against Red Flour Beetle (*Tribolium castaneum*)

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Abstract: Efficacy of neem (*Azadirachta indica*) and acacia (*Acacia auriculiformis*) extract was assessed on the basis of toxicity and repellency effects by red flour beetle. The study followed a completely randomized design with three replications. Plant extracts concentrations were 5%, 7.5%, and 10% and control (only solvent treated). The results revealed that neem leaf extract, particularly at 10%, exhibited the highest efficacy, showing 53.94% adult mortality and 49.99% repellency against red flour beetle. In contrast, 5% of the acacia leaf extract demonstrated the lowest mortality (23.09%) and repellency (26.53%). The effectiveness of both extracts increased with higher concentrations, neem showing superior activity compared to acacia. These findings suggest that neem leaf extract, especially at higher concentrations, could serve as a promising, eco-friendly alternative for controlling red flour beetle infestations in stored grains.

Key words: Efficacy of neem extract; Acacia extract; Red flour beetle.

INTRODUCTION

The red flour beetle is a serious insect pest not only of maize but also of all cereals and their products worldwide. A significant portion of the world's agricultural output is lost as a result of stored grain insect infestations after harvest, which has a negative impact on the economy and the availability of food. Insect pests cause significant losses in stored cereal grains worldwide, with an estimated 10-20% yield reduction (Haque *et al*. 2000; Tripathi *et al*. 2001; Rajendran and Sriranjini 2008). Specifically, pest infestations in storage alone cause a yearly productivity loss of about 8%. Some studies indicate that potential losses range from 6-18% annually and in maize even up to 80% within 5-6 months of storage (Alam *et al. 2019)*. These losses highlight the urgent need for effective pest management strategies, including improved storage practices to ensure food security and economic stability.

Traditionally, chemical insecticides and fumigants have been the primary means of controlling storage pests. Prolonged and indiscriminate use of chemicals has led to serious issues including pesticide resistance, environmental pollution, toxic residues in food and adverse health effects (Pimentel, 2005; Arthur, 2016). Additionally, the rising costs of synthetic insecticides have made them less accessible to farmers, particularly in developing countries (Subramanyam & Hagstrum, 2012). Botanical pesticides have gained attention as biodegradable, environmentally safe and non-toxic alternatives to synthetic chemicals, offering a sustainable approach to pest management (Mordue & Nisbet, 2000; Pavela & Benelli, 2016).

Botanicals of various kinds have been noted for their insecticidal efficacy and they are being regarded as better alternatives to synthetic chemical insecticides (Scott *et al.* 2003*).* Therefore, many researches have been done to screen many plant species for their insecticidal potential because they are believed to contain numerous phytochemicals that could have antifeedant and considerable toxicity against insects (Berenbaum and Zangerl 1996; Zibaee 2011; Ashamo *et al* 2013). Despite the effectiveness of many botanicals there is still a wide gap between them and synthetic chemical insecticides as many of them loss their potency over time (Forim *et al.* 2012). So, there is a need to evaluate other botanicals that could comparably contend with synthetic chemical insecticides. The purpose of the study was to find out the effectiveness of locally available plants namely, Acacia (*Acacia auriculiformis*) and Neem (*Azadirachta indica)* against red flour beetle for their insecticidal properties.

MATERIALS AND METHODS

Insect rearing

The test insect species red flour beetle, *Tribolium castaneum* (Herbst) was collected from Bangladesh Institute of Nuclear Agriculture (BINA) in Mymensingh and was maintained in the laboratory of the Department of Entomology, BAU, Mymensingh at 27-30°C and 70-75% RH. Adult red flour beetles were reared on fresh wheat flour. Wheat flour was free from any chemical treatment. This wheat flour is used in rectangular jars (9.5 cm x 7.5 cm) and varieties of jar and pots. Each jar was set up with 10 pairs of adult red flour beetles. The mouth of the jar and pot was covered with a piece of cloth fastened with rubber band to prevent contamination and insect escape and these jar and pot are kept on table in the laboratory without any disturbance.

Plate 1. Collection and rearing of red flour beetle

Collection and processing of plant materials

Fresh leaves of acacia, *Acacia auriculiformis* and neem, *Azadirachta indica* were washed in running water and then they were dried under sun for fine dust. Sun dried leaf dust was prepared by pulverizing the dried leaves in a magnetic stirrer. A sieve was used to obtain fine & uniform dust and preserved them into an air tight plastic jar, till their use in extract preparation.

Preparation of plant extracts

At first 50g of each category of dust were taken in a 500 ml beaker and separately mixed with 300 ml of 70% methanol solvents. Then the mixture was stirred for 15 minutes by a magnetic stirrer and was kept standing for the next 24 hours. The mixture was then filtered through a fine cloth and again through filter paper (Whatman No. 1). The filtered materials were taken into a round bottom flask and then condensed by evaporation of solvent in a water bath at 55°C temperature. After the evaporation of solvent from filtrate, the condensed extracts were preserved in tightly corked-labeled bottles and stored in a refrigerator until their use for insect bioassays.

Plate 2. Preparation and filtration of extracts

Direct mortality test

Ten insects per treatment were treated and each treatment was replicated 3 times. In addition, the same numbers of insects were treated with solvent only for control. Insects were examined daily and those that did not move or respond to gentle touch were considered as dead. Insect mortalities were recorded at 24, 48 and 72 hours after treatment (HAT).

Plate 3. Mortality test of red flour beetle Observed mortalities of the insects were corrected by Abbott's formula (1987): Corrected mortality (%) = (Observed mortality–Control mortality/100 - Control mortality) x 100.

Repellency Test

Nine centimeter diameter petri dish was divided into three portions, treated and untreated grain portion 3.5 cm each and neutral center portion (without grain) 2 cm. Two grams of wheat grain were taken in each side portion of petri dishes for rice weevil. 1 ml of solution of different doses of each plant extract was applied to a side portion as uniformly as possible with a pipette i.e. treated grain side portion and the other side was control side. Ten insects were released at the central portion of each petri dish and a cover was placed on the petri dish. There were three replications for each plant leaf extract and for each dose. In the control side portion of the petri dish the grains were treated with solvent only. Then the number of insects on each portion was counted at hourly intervals up to the 5th hour.

Plate 4. Repellency test of plant extracts on red flour adults using treated wheat grain

The data were converted to express percent repulsion (PR) by the following formula (Talukdar and Howse, 1994).

$$PR\ (\%) = (Nc-50) \times 2$$

Where, Nc= the percentage of insect present in the control half.

Positive values express the degree of repellency and negative values for the level of attractancy. Data PR (%) were analyzed using ANOVA. The average values were then categorized into classes (McDonald et al., 1970) viz., 0=>0.01-0.1%, I=0.1-20%, II=20.1- 40%, III=40.1- 60%, IV=60.1- 80% and V=80.1-100% repellency rate.

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Results And Discussion

Leaf extracts from two native plants were tested against the red flour beetle. Under the following subheadings, the findings from a variety of experiments carried out during the research period (March-October'24) are shown and discussed.

Effects of methanol extracts on red flour beetle

The adult mortality rate of red flour beetle, *Tribolium castaneum* treated with methanol extract of acacia and neem leaf at various doses under laboratory conditions is presented in Table 1 and Fig 1.

Table 1 showed the effects of plant leaf extract, dosage and time. Mortality percentages at 24 hours after treatment (HAT) revealed that the 10% neem leaf extract had the highest toxic effect (39.25%) on red flour beetle followed by the acacia extract (31.85%). A similar pattern was observed at 48. Neem extract showed the highest toxicity effect (66.19%) than acacia (52.68%) at 72 HAT. The 7.5% neem leaf extract showed moderate toxicity (28.14%) on red flour beetles with acacia extract causing (17.40%) mortality at 24 HAT. This pattern remained consistent at 48 and 72 HAT. The 5% neem leaf extract exhibited the lowest toxicity (20.37%) on red flour beetles, followed by the acacia extract (13.33%) at 24 HAT, with the same trend observed at 48 and 72 HAT.

Table 1. Effects of two plant leaf extracts at different dose level of the mortality on red flour beetle

Name of the extracts	Dose (%)	Adult mortality (%)		
		24 HAT	48 HAT	72 HAT
Neem	5	20.37	33.097	42.96
	7.5	28.14	42.76	52.85
	10	39.25	56.39	66.19
Acacia	5	13.33	23.06	32.89
	7.5	17.40	25.92	39.39
	10	31.85	42.92	52.68
Sx̄		3.34	2.56	3.01
Probability level		NS	NS	NS

NS= Not significant
HAT= Hour after treatment

The average mortality percentages of red flour beetles at 24, 48, and 72 hours after treatment (HAT) showed that the 10% neem extract had the highest toxic effect (53.94%), while the 5% acacia leaf extract exhibited the lowest toxicity (23.09%), as shown in Fig. 1. The toxicity order of the two plant leaf extracts on red flour beetles was: neem > acacia. Mortality percentages increased in direct proportion to the time after treatment.

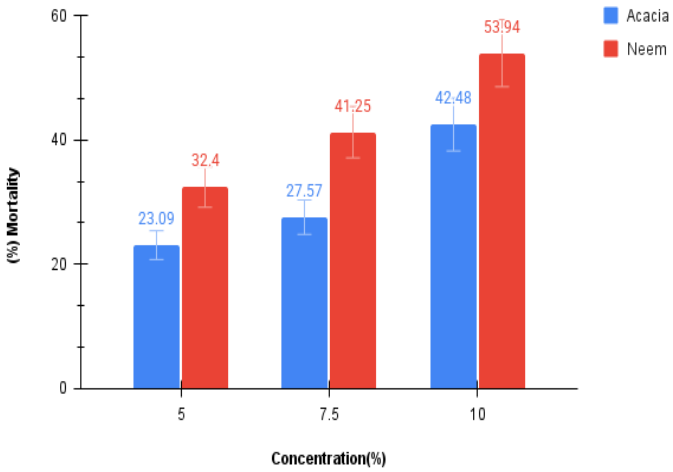

Fig 2. Effects of neem and acacia leaf extracts at different dose level on % mortality of red flour beetle

The findings in this study are consistent with those of Siddig, S.A. *et al.* (1991), who observed that *A. indica* exhibited toxicity toward Coleopterous insects. Similar results were reported by Elzaki *et al.* (2014), who highlighted the insecticidal potential of *A. indica* against *T. castaneum*. Additionally, the findings support the work of Ahmad *et al.* (2019) and Kumar *et al.* (2023), which showed that neem significantly reduced adult beetle emergence and grain loss across various commodities. The effectiveness of neem is primarily attributed to azadirachtin, a compound that interferes with hormonal processes, feeding behavior and reproduction.

The results also align with those of Kaur *et al.* (2009), who found that *A. auriculiformis* exhibits notable anti-insect properties. These findings are further supported by Tedela *et al.* (2017), who emphasized the plant's significant insecticidal potential. Although acacia showed somewhat lower effectiveness, it still demonstrated notable insecticidal activity, consistent with the observations of Alves *et al.* (2019), who reported a strong dose-dependent response of acacia against *T. castaneum,* with mortality rates reaching high levels at a 5% concentration.

Repellent Effect on red flour beetle, *Tribolium castaneum*

The results of the repellency effect of methanol leaf extracts of acacia and neem on red flour beetles are presented in Table 2. Both acacia and neem leaf extracts exhibited repellent action, with their effects at different hours being statistically not significant ($P<0.01$). The highest repellency (49.99%) was observed with the 10% neem extract, compared to acacia (43.33%), both belonging to repellency class III. The lowest repellency rate (26.53%)

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was recorded for the 5% acacia leaf extract, which was lower than that of the neem treatments.

The results in both cases indicated that the repellency effect increased in proportion to the concentration. Of the two leaf extracts, neem exhibited a more pronounce repellency effect compared to acacia.

Table 2. Repellency effect of neem and acacia methanol leaf extracts at varying concentrations on *Tribolium castaneum*

Name of the plant leaf extracts	Dose (%)	Repellency rate (%)					Mean repellency rate (%)	Repellency class
		1HAT	2HAT	3HAT	4HAT	5HAT		
Neem	5	30.00	33.33	43.33	36.67	53.33	39.19	ii
	7.5	33.33	46.67	46.67	40.00	56.67	44.67	iii
	10	43.33	46.67	53.33	43.33	63.33	49.99	iii
Acacia	5	16.00	23.33	26.67	30.00	36.67	26.53	ii
	7.5	26.67	36.67	33.33	40.00	43.33	36.00	ii
	10	33.33	43.33	46.67	40.00	53.33	43.33	iii
Sx̄		2.54	4.19	3.04	3.33	3.47		
Probability level		NS	NS	NS	NS	NS		

NS = not significant; HAT = Hours After Treatment

The repellency rate increased with extract concentration, and neem remained more effective than acacia across all levels tested. These findings are supported by Hassan and Drees (2005), who observed that neem-treated flour was significantly attractive to *T. castaneum* and showed a clear dose-dependent repellency. Kumar *et al*. (2023) also found neem to be the most effective botanical in repelling *T. castaneum*, achieving the highest repellency rates among several tested species. Acacia showed moderate yet consistent repellency against red flour beetles. Suthisut *et al.* (2012) linked Acacia's bioactivity to its polyphenolic profile, which disrupts feeding behavior and sensory responses in *T. castaneum*. In addition, Tedela et al. (2017) observed similar effects, reinforcing its potential as a botanical insect repellent. Following comprehensive analysis, *A. indica* extract proved to be more potent than *A. auriculiformis* in repelling *T. castaneum*, with its effectiveness likely attributed to active compounds such as azadirachtin. Nevertheless, *A. auriculiformis* also exhibited consistent, concentration-dependent repellency, supporting its potential role in eco-friendly pest management strategies for stored grains.

CONCLUSION

Botanical products are effective, easy to handle and low cost to prepare and plants are available around us. Each year Bangladesh imports a huge quantity of insecticides to minimize the severe damage caused by insect pests. As our farmers are poor, they cannot spend a huge amount of money on synthetic insecticides to protect their stored products. So, they can use botanical materials as insecticides, which will benefit our agricultural sector as these substances are not only of low cost but also have less environmental impact in terms of insecticidal hazards. The findings of the present study indicated that *A. auriculiformis* and *A. indica* can be utilized as effective insect-controlling agents in storage conditions, provided they are used at appropriate concentrations and doses. The results demonstrated the potential of these two plant products in managing insect pests during storage. In comparison, the experimental findings, supported by extensive literature, confirm that neem leaf extract is a superior botanical pesticide, particularly when compared to acacia, showing higher efficacy in terms of both mortality and repellency against *T. castaneum*. Neem's effectiveness is attributed to its rich phytochemical profile, especially azadirachtin, which disrupts insect physiology, feeding, and reproduction.

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Conflict of Interest

The authors declare that there is no conflict of interest among authors regarding the submission and publication of this manuscript.

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