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Insecticidal effect of botanical oils for protection of wheat seeds against red flour beetle (*Tribolium castaneum*)

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General Note
Article is recommended to print as color version in recycled paper. *Save Trees, Save Nature*.

ABSTRACT

Botanical oil is an effective element to control stored grain pest like red flour beetle. The main purpose of this study was to determine the direct toxic effects of the different plant oils. This study used five indigenous plant's seed oil to explore the toxicity level of the plants against red flour beetle. These used plants were Karanja (*Pongamia pinnata*), Mahogoni (*Swietenia mahagoni*), Neem (*Azadirachta indica*), Pithraj (*Aphanamixis polystachya*) and Sesame (*Sesamum indicum*). This study also used a germination test of wheat seed to explore the effectiveness of the botanicals. This study revealed that Sesame oil showed the most toxic and repellent effect. The result showed that all the plant oils did not show any adverse effect on germination capability of seeds up to 90 days of treatment. All these indigenous plants are available throughout the country so farmers can easily use these plants in their storage structure for management of stored grain pests.

Keywords: Indigenous plant, botanical effect, toxicity, bio-control, red flour beetle.

1. INTRODUCTION

Wheat is the second most important cereal crop next to rice in Bangladesh and has gained much popularity among the farmers due to its higher nutritive value and lower cost of production than rice (Sarker et al., 2015; Sarker, 2016a; Sarker, 2016b; Sarker et al., 2017; 2017; Sarker et al., 2019). Losses occurred due to insect infestation in storage are the most serious problem in grain storage, particularly in villages and towns in tropical and subtropical countries, because of humid conditions, poor sanitation and inappropriate storage facilities (Salunke et al., 2009; Singh & Kaur, 2018). In tropical countries like Bangladesh, the climate and storage conditions are highly favorable for insect growth and development (Talukder & Howse, 1994; Sarker et al., 2007; Ali, et al., 2015; Haider et al., 2015; Islam et al., 2018; Islam et al., 2018; Sarker, 2017; Sarker, 2016d; Sarker, 2016b; Sarker et al., 2015; Sarker, 2019). About 600 species of insects belonging to different families have been identified from stored products in various parts of the world. It has been estimated that about 15-20% of the worlds agricultural production is lost each year due to insect infestation. Out of this, 8% of production is lost each year due to insect infestation alone in storage (Islam et al., 2015a). If the losses incurred on farms were included, it would amount to 10%. If this 10% could be saved from destruction and properly distributed, famine could be averted in the developed countries of Asia and Africa (Wagan et al., 2016). The stored product insects not only feed on the stored food but also cause organoleptic changes in food grains, as a result, the nutritional value of the food grains is reduced (Adarkwah et al., 2010; You et al., 2017). In addition, they also cause fermentation and acidification of food grains and many other changes. Sometimes molds grow in the insect-infested food grains and these molds produce a chemical substance called aflatoxin which is reported to be associated with the lever cancer of human being. There are over' 200 major species of insects and mites infesting important crops and stored products. Among these species, red flour beetle, *Tribolium castaneum* is a common and most destructive pest (Muhammad Bilal Saleem et al., 2018). It is very common and cosmopolitan in distribution. This insect is found around the year in stored wheat and wheat flour but the intensity of infestation is higher in summer than in winter due to high temperature and relative humidity (Ahmad et al., 2018). Red flour beetle is a serious pest and occurs widely throughout the world (Md Shahidul Islam, Ali, & Sarker, 2015b). Both the adults and grubs cause serious damage to all kinds of grains including flour, dried fruits, and many other foods. This pest is generally found in granaries, mills, warehouse, etc., feeding on rice (both husked and unhusked).

At present different kinds of preventive and curative control measures are practiced to protect this pest. Among those, chemical pesticides have been used for a long time, but they have serious drawbacks (Hashem et al., 2018). Control of insect pest by the routine use of chemical insecticides created several problems in agro-ecosystem such as direct toxicity to beneficial insects, fishes and man, pesticides resistance, a health hazard, and increased environmental and social costs. Sometimes persistent pesticides accumulate in the higher food chain of both wildlife and human and become concentrated by bio-magnification. The development of cross and multi-resistant strains in many important insect species, resulting from the continuous use of chemical insecticides has been reported from all over the world (Taban et al., 2017).

In many countries, efforts are being made to minimize the use of harmful insecticides through the use of indigenous plant products, implementation of IPM approaches, use of bio-degradable products and applying insect growth regulators to protect stored grains. In many areas of the world locally available plant materials are widely used to protect the stored product against damage by insect infestation. These plant products are environmentally safe, less hazardous, less expensive and easily available. Plant product has defense compounds which make difficult or impossible for pests to feed and would neither change the taste or smell of the stored product nor threaten the environment or consumer (Sarker et al., 2018; Sarker et al., 2019). There are about 2000 plant species seems to possess pest control. Botanicals like Karanja, Mahogoni, Neem, Pithraj, and Sesames are grown by farmers with minimum maintenance and extracted by indigenous methods (Zaka et al., 2018). The main advantage of botanical insecticides is that they can be easily produced by the farmers in the house and in small industries. However, very few scientific research works have been done in Bangladesh to explore our locally available plant materials for the control of harmful insect pests in storage. These botanical materials can be used as an alternative to chemical pesticides. This will be very helpful in minimizing the undesirable side effects of synthetic pesticides. The present study was carried out with five indigenous plant seed oils namely Karanja (*Pongamia pinnata*), mahagoni (*Swietenia mahogany*), Neem (*Azadirachta indica*), Pithraj (*Aphanamixis polystachya*) and Sesame (*Sesamum indicum*) to evaluate their toxic effects against a major stored product insect-like red flour beetle (*Tribolium castaneum)* and also their effect on germination capability of treated grains. The main purpose of the present research was to determine the direct toxic effects of the different plant oils and to observe the germination feature of treated wheat seeds.

2. MATERIALS AND METHODS

The present study was conducted to evaluate some indigenous plant oils viz. Karanja (*Pongamia pinnata*), Mahogoni (*Swietenia mahagoni*), Neem (*Azadirachta indica*), Pithraj (*Aphanamixis polystachya*) and Sesame (*Sesamum indicum*) against red flour beetle, *Tribolium castaneum* Herbst. In the laboratory of the Department of Entomology, Bangladesh Agricultural University, Mymensingh, Bangladesh. Selected plant oils were tested to determine their toxic effects and to observe the germination feature of treated wheat seeds.

2.1. The test plants

Five indigenous plants were selected for the experiment which included karanja mahogoni, neem, pithraj and sesame. The seed oil of the plants was used for this experiment. These were collected from different areas of Mymensingh, Bangladesh. The short descriptions of the plants are presented in Table 1.

Table 1 Short descriptions of the plants

Plants for seed oils	Scientific name	Family	Used parts
Karanja	*Pongamia pinnata*	Leguminosae	Seeds
Mahogoni	*Swietenia mahagoni*	Meliaccae	Seeds
Neem	*Azadirachta indica*	Meliaceae	Leaves
Pithraj	*Aphanamixis polystachya*	Meliaceae	Seeds
Sesame	*Sesamum indicum*	Pedaliaceae	Seeds

2.2. The test insect

The study was conducted with a major stored gain insect pest, the red flour beetle, *Tribolium castaneum* Herbst. It belongs to the family Tenebrionidae under the order Coleoptera. The adults are 2.3-4.4 mm in length and 1.2 mm in width and red-brown in color. The species is similar in appearance to *Tribolium confusum* but may be distinguished from it by certain features of the eyes and antennae. The antenna is clavate with three apical segments suddenly enlarged. The distance between the two compound eyes ventrally is three times the diameter of each eye. The male possesses a hairy puncture on the ventral surface of the anterior femur, this puncture is absent in the female. Though the red flour beetle has been originated in India, it is found all over the tropical, subtropical and warm temperate regions of the world. It is one of the most common pests of stored products. The insect breeds from April to October and passes the winter mostly in the adult stage. The females lay eggs in the commodity throughout their adult life. The number of eggs laid depends on temperature; on an average, they lay 2.5 eggs/day at 25°C but it rises to 11 eggs/day at 32.5°C. Up to 300-400 eggs may be laid at random in the flour or in the brassy material among the grains and other food stuff. The surface of the freshly laid eggs is sticky and therefore, flour or dust particles easily adhere to them. The incubation period lasts for 4-10 days. Small white eggs hatch into worm-like larvae which are slender, cylindrical and wiry in appearance. The larvae undergo 6-7 molting and become full-grown in 22-25 days at 30°C. Pupation takes place in the flour. The pupa is yellowish and hairy and the pupal stage lasts for 5-9 days. The developmental period from egg to adult may be as short as 28 days but as usually longer when conditions are unfavorable and the available food is unsuitable. The optimum conditions for the development of this pest are 30°C and 70% relative humidity. Adult beetles have been known to live for as long as 1.5 years (Plate 3.4). The larvae and adults feed on a wide range of durable commodities and important secondary pests of cereals, nuts species, dried fruits and occasionally of pears and beans. Like most other storage beetles, *T. castaneum* can penetrate deeply into the storage commodity. However, the red flour beetle can only attack the broken grains and therefore, they are known as secondary stored product pests.

2.3. Collection and rearing of insect

The test insect species, *T. castaneum* was collected from food stores of Mymensingh city, Bangladesh and were maintained in the laboratory of the Department of Entomology, BAU, Mymensingh, at 27-30˚C and 70-75% RH. The insects were reared on fresh wheat grains in rectangular jars (9.5 cm x 7.5 cm). Each jar was set up with 20 pairs of adult insects. The mouth of the jar containing wheat grain was covered with a piece of cloth fastened with a rubber band to prevent contaminations and insect escape. After 7 days, all the adults of red flour beetle were removed from each jar and jars were left undistributed for completing the life cycle of the insect. The first generation emerged within 28 days after the removal of parent stocks. These newly emerged adults were utilized in the present experiment.

2.4. Collection and processing of plant materials

Fresh seeds of karanja, mahogoni, neem, pithraj and sesame were collected from the surroundings of BAU Campus, Mymensingh. After bringing them to the laboratory, they were washed in running water. Firstly the plant materials were kept in shade for air-drying and then they were dried in the oven at 60°C to gain constant weight.

2.5. Preparation of oils

Four kg fresh seeds of karanja, mahogoni, neem, pithraj, and sesame were taken for extraction of oil. Oil extraction was then done by oil extraction machine and fresh oil was preserved in glass bottles.

2.6. Treatment

Five treatments including control were done. The treatments included the application of oils of karanja, mahogoni, neem, pithraj, and sesame mixed with wheat seeds @ 3.0, 4.0, 5.0, 6.0 percent and an untreated control. Each treatment along with control was replicated thrice.

2.7. Disinfestations of Wheat grains

The wheat grains were spread on black polythene sheet and sun-dried from 10.00 A.M. to 2.30 P.M. in direct sun light with air temperature ranging from 30-40°C for five consecutive days. Sun-dried grains were kept in the laboratory for few hours packed in a polythene bag and sealed to avoid future infestation. Fresh and disinfested wheat grains were used for the experiments.

2.8. Insect bioassays

A study was designed with following types of bioassays to determine the toxicity, repellency and grain protection efficacy against red flour beetle *T. castaneum*.

2.9. Toxicity test

The toxicity test was conducted according to the method described by Talukder and Howse (1994) with slight modification. At first, oil (3.0, 4.0, 5.0 and 6.0%) was mixed with 10 g wheat grains and then treated grains were placed in sterilized Petridis (9 cm diameter). Five pairs of adult insects were taken in each Petridis and covered. Five treatments along with control and three replications were made for each treatment. Insect mortalities were recorded al 24, 48, 72 and 96 hours after treatment (Figure 1).

Figure 1 Showing Toxicity test

2.10. Seed germination test

Wheat seeds of the variety "Shourov" were collected from the Wheat Research Centre (WRC), Bangladesh Agricultural Research Institute. The seeds were treated with 3.0, 4.0, 5.0 and 6.0% solution of the plant oils. The seeds were then shade dried and kept for 3 months with a sealed poly bag to prevent infestation. Control wheat seed was also kept in the same way.

Figure 2 Shows germination test

The seeds were then taken to test their germination quality by using blotting paper. The well-germinated seeds in each petridishes were counted after 7 days of setting experiment. Germination percentage was determined on filter paper in petridishes (Figure 2).

2.11. Statistical analysis

The observed values obtained during the experiments were transformed where necessary and the values were analyzed by factorial completely randomized design (CRD). Mean values were adjusted by Duncan's multiple range test (DMRT). For mortality test, original data were corrected by Abbott (1987) formula as follows:

$$\text{Corrected Mortality} = \frac{\text{Observed mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 100$$

LD50 values were calculated by using Probit analysis.

3. RESULTS AND DISCUSSION

The effect of some indigenous plant seed oils was evaluated against red flour beetle, *Tribolium castaneum* Herbst. The results of various experiments conducted during the study period are discussed below:

3.1. Toxicity test

The results of the toxic effects, both individual and combined effect of karanja, mahogoni, neem, pithraj and sesame oils on the red flour beetle, *T. castaneum* are presented in Tables 4.1-4.3. The doses used were: 3.0, 4.0, 5.0 and 6.0%. Average mortality percentage of red flour beetle at 24, 48, 72 and 96 hours after treatment (HAT) indicated that sesame oil possessed highest (53.17%) toxic effect, whereas karanja oil possessed the lowest (19.54%) toxic effect, in case of individual oils. On the other hand, pithraj + sesame oil possessed the highest (51.67%) toxic effect, whereas karanja+mahogoni oil possessed the lowest (21.84%) toxic effect in case of combined oils (Table 2). The differences among different oils were significant statistically. The order of toxicity of the five plant oils on red flour beetle, *T. castaneum* were: sesame >pithraj> neem >mahogoni>karanja, for individual oil and pithraj + sesame > sesame + karanja>mahogoni+neem> neem + pithraj>karanja + mahogoni for combined oils.

Table 2 Mean mortality percentage of the red flour beetle in wheat grain treated with different plant seed oils at different HAT (Interaction of oil and time)

Name of the plant seed oils	Percentage of insect mortality at				The average value (%)
	24 HAT	48 HAT	72 HAT	96 HAT	
Karanja	12.67 q	18.00 o	22.67 n	24.80 n	19.54 i
Mahogoni	14.67 p	18.67 o	21.73 n	24.67 n	19.94 h

Neem	44.67 hi	48.00 g	52.67 d	54.00 cd	49.84 d
Pithraj	47.33 g	52.00 de	56.00 b	57.34 a	53.16 a
Sesame	48.67 g	52.67 d	54.67 c	56.67 ab	53.17 a
Karanja + Mahogoni	15.33 p	20.67 n	24.67 m	26.67 l	21.84 g
Mahogoni+Neem	43.33 hi	46.67 h	48.00 g	50.00 e	47.00 e
Neem + Pithraj	36.67 k	41.99 j	45.33 hi	46.00 h	42.50 f
Pithraj+ Sesame	46.00 hi	50.67 e	54.00 cd	56.00 b	51.67 b
Sesame + Karanja	44.00 hi	49.33 f	52.67 d	56.00 b	50.50 c
S$\bar{x}$	0.4785				0.5634
Probability level	0.01				0.01

Note: *HAT = Hours after treatment*

Within column values followed by a different letter(s) are significantly different by DMRT. The highest average mortality (73.65%) was observed at the highest concentration (6%) of plant oils and it was also found that the mortality percentage was directly by proportional to the level of concentration of plant oils. The interaction effects of plant oil, dose and time are presented in Table 4.3. The interaction effects of the plant, dose and time had no significant effect on the mortality of *T. castaneum* except their average values. Here the highest (92.50%) average mortality was found from the sesame oil at 6.0% and lowest (9.17%) from the mahogoni oil at 3.0% concentration level. Similar result was found by Ahad et al. (2018) in case of jute.

3.2. Probit analysis for toxic effect

The results of the probit analysis for the estimation of LD_{50} values, 95% fiducial limits and the slope of regression lines at 24, 48, 72 and 96 HAT for the mortality of red flour beetle are presented in Table 4.4. The LC_{50} values for karanja, mahogoni, neem, pithraj and sesame at 24 HAT (Table 4.4) indicated that sesame (1.66%) was the most toxic and mahogoni (8.93%) was the least toxic, in case of individual oil. In the case of combined oils, pithraj + sesame (1.87%) was the most toxic and karanja plus mahogoni (5.17%) was the least toxic. At 48 HAT, comparison of LC_{50} values showed that sesame oil possessed the highest toxic effect (1.41%), whereas mahogoni possessed the lowest (7.49%) toxic effect, in case of individual oil. On the other hand, in case of combined oil pithraj + sesame possessed the highest toxic effect (1.67%) and karanja + mahogoni possessed the lowest toxic effect (5.63%).

When LC_{50} values were compared at 72 HAT, it was found that pithraj oil showed highest toxicity (1.27%) and karanja showed lowest (6.32%) toxicity, in case of individual oils. In case of combined oils, sesame + karanja showed highest toxicity (1.40%) and karanja + mahogoni showed lowest toxicity (4.85%).

3.3. Germination effect

Results on the individual and combined effect of different plant seed oils such as karanja, mahogoni, neem, pithraj, and sesame on the germination percentage of wheat seeds are presented in Table 3. Interaction of different plants oils at different dose level had a significant effect on germination of wheat seeds. In the case of individual oils, on the basis of average values, the highest germination (94.72%) was observed in wheat seed treated with karanja oil and lowest (92.76%) in pithraj oil. On the other hand, incase of combined oils, the highest germination (93.61%) was observed in sesame + karanja oil and lowest (92.22%) in mahogoni + neem and neem + pithraj oil. Different plant seed oils had a significant effect on germination of wheat seeds (Table 3).

Table 3 The germination rate of wheat seeds treated with different plant seed oils (Interaction of oil and dose)

Name of the plant seed oils	Germination percentage				The average value (%)
	Doses %				
	3.0	4.0	5.0	6.0	
Karanja	95.56 a	93.33 c-f	95.56 a	94.44 b-d	94.72 a-c
Mahogoni	95.56 a	94.44 b-d	94.44 b-d	93.33 c-f	94.44 b-d
Neem	94.44 b-d	92.22 e-h	93.33 c-f	92.22 c-h	93.05 c-f
Pithraj	93.33 d-g	93.33 c-f	92.22 e-h	92.22 e-h	92.76 c-e
Sesame	94.44 b-d	92.22 e-h	93.33 c-f	91.11 g-i	92.78 c-e
karanja + mahogoni	94.44 b-d	93.33 c-f	91.11 g-i	92.22 e-h	92.77 c-e
Mahogoni + neem	92.22 e-h	93.33 c-f	92.22 e-h	91.11 g-i	92.22 e-h

neem + pithraj	93.33 c-f	92.22 e-h	93.33 c-f	90.00 h-j	92.22 e-h
pithraj + sesame	94.44 b-d	93.33 c-f	91.11 g-i	92.22 e-h	92.78 c-e
sesame + karanja	95.55 a	92.22 e-h	93.33 c-f	93.33 c-f	93.61 c-f
$S\bar{x}$	0.1920				0.0972
Probability level	0.05				0.01

NS = Not significant

Within column values followed by a different letter(s) are significantly different by DMRT. The present experiment was carried out in the Laboratory of the Department of Entomology, Bangladesh Agricultural University, Mymensingh. The plant seed oils of karanja (*Pongamia pinnata*), mahogoni (*Swietenia mahagoni*), neem (*Azadirachta indica*), pithraj (*Aphanamixi spolystachya*) and sesame (*Sesamum indicum*) at 3.0, 4.0, 5.0 and 6.0% concentrations were evaluated for their toxic, repellent and grain protectant effects against red flour beetle, *Tribolium castaneum* Herbst. Germination capability of treated seeds was also tested. Similar result was found by Islam et al. (2015) in case of wheat.

In case of individual oil, mortality percentage of red flour beetle at 24, 48, 72 and 96 HAT indicated that sesame oil possessed the highest toxic effect followed by pithraj, neem, mahogoni, and karanja. On the other hand, incase of combined oils Pithraj + Sesame possessed the highest toxic effect followed by Sesame + Karanja, Mahogoni + Neem, Neem + Pithrajand Karanja + Mahogoni. Mortality percentage may found to be directly proportional to the level of concentration of plant oil. The LC_{50} values of karanja, mahogoni, neem, pithraj and sesame at 24 HAT indicated that sesame oil was the most toxic (1.66%) in case of individual oil and pithraj + sesame was the most toxic (1.87%) in case of combined oils. It was also observed that sesame (in case of individual oil) possessed the highest toxic (1.41%) and Pithraj+ sesame (in case of combined oil) possessed the highest toxic (1.67%) effect at 48 HAT. At 72 HAT Pithraj was most toxic (1.27%) in case of individual oil and Sesame + Karanja was most toxic (1.40%), incase of combined oil. It also observed the same trend of results was observed at 96 HAT.

Mean values indicated that the germination percentage of treated seed with different plant seed oils at different dose level varied significantly. Incase of individual oils, the highest germination was found in karanja oil treated wheat seeds and lowest in pithraj oil treated wheat seeds. The results also showed that all the treated seeds were not equally viable upto 90 days of treatment. Germination of wheat seeds decreased gradually with the increase of doses (Ahad et al., 2019).

4. CONCLUSION

Laboratory experiments were carried out to determine the toxicity and seed germination effect of Karanja, Mahogoni, Neem, Pithraj and sesame oils. Good evidence was obtained from the present experiment among the plant oils. Sesame oil showed the most toxic and repellent effect. The result showed that all the plant oils did not show any adverse effect on germination capability of seeds up to 90 days of treatment. Sesame and Pithraj are available throughout the country. Farmers may use those plants in their storage structure for management of stored grain pests. But before releasing botanical material as new technology it may further be verified in limited farmer's storage system. Each year Bangladesh imports a huge quantity of insecticides to minimize the severe damage caused by insect pests. Since farmers of Bangladesh 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 a less environmental impact in terms of insecticidal hazards. The botanical products are effective, easy to handle and low cost to prepare and plants are available around us. Therefore, the use of botanical materials in Bangladesh will be highly effective against stored product insect pests and may be suggested to be used by the farmers of Bangladesh. Further research may be conducted to isolate the active ingredients present in the seed soils and these may be released as a botanical insecticide against stored grain insect pests, especially red flour beetle.

Authors' contributions

All authors contributed equally from research design to manuscript preparation. All authors checked the final manuscript and approved for publication.

Disclosures about potential conflict of interests

All the authors declare that there is no potential conflict of interest among the authors.

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