Pesticidal Evaluation of *Manilkara zapota* (L.) against *Tribolium castaneum* (Herbst)

Muhammad ABU OSMAN
Muhammad ABDUL AZIZ
Muhammad ROWSHANUL HABIB
Muhammad REZAUL KARIM (✉)

Summary

This paper presents the residual effects of *Manilkara zapota* (L.) P. Royen plant against the red flour beetle, *Tribolium castaneum* (Herbst). Four doses, i.e., 1238.5, 619.25, 309.6 and 154.8 μg/cm^2 of ethyl acetate extract of stem bark of *Manilkara zapota* were applied on larvae and adult beetles. The effectiveness of the plant extract was increased with the increase of exposure time and after 72 hrs of exposure, the maximum residual toxicity was observed with LD_{50} of 228.8, 281.1, 413.4, 423.7, 455.2, 498.7 and 526.5 μg/cm^2 for first, second, third, fourth, fifth, sixth instar larvae and adults, respectively. The results of this study also demonstrated that the earlier instars were more sensitive to the extracts than those of late instars as well as adults.

Key words

Manilkara zapota, *Tribolium castaneum*, Ethyl acetate extract, Stem bark

[1] Rajshahi University, Department of Biochemistry and Molecular Biology, Rajshahi-6205, Bangladesh
✉ e-mail: rezaplazad@yahoo.com

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Introduction

Insect infestation of stored grains and their products is a serious problem throughout the world (Irshed and Gillani, 1990). In tropical countries like Bangladesh, the climate and storage conditions are favorable for insect growth and development (Talukder and Howse, 1995). The world's cereal production is lost up to 10% every year due to insect infestation in storage (Wolpert, 1967). Today, losses from storage pests cannot be measured only by the amount of food or seeds destroyed by insects. The mere presence of insect fragments in food is objectionable to most consumers. Thus economic losses are far more due to presence of residues and contamination of food materials and it is a highly objectionable and notable problem. Losses due to insect damage are not so serious but it is the incorporation or addition of non-degradable pesticidal contaminants in the food, feed and in ecological surroundings. The economic losses may be result from insect contamination, although actual losses of food materials due to insect feeding may be negligible (Kundu, *et al*., 2007). Among the important stored-product insect pests, the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is common and most destructive pest throughout the world. This pest has been reported to attack the germ part (embryo portion) of the grain. Their presence in stored foods directly affects both the quantity and quality of the commodity (Mondal, 1994). Currently different kinds of preventive and curative control measures are practiced to get protection from this pest. Among those, chemical pesticides have been used for a long time, but have serious drawbacks, such as direct toxicity to beneficial insects, fishes and humans due to their effects on non-target organisms (Matthews, 1993; Rajeskaran and Baker, 1994 and Gupta *et al*., 2001). Due to the problems associated with the indiscriminate use of synthetic insecticides like insect resistance and impact on non-target organisms, many scientists throughout the world have concentrated on the search for active natural products derived from plants as ecologically safe alternatives, because globally there is growing awareness and desire to utilize natural and environment-friendly compounds for pest control (Isman, 2000; Kim *et al*., 2003; Mehmet *et al*., 2003; Sahayaraj *et al*., 2003; Bughio and Wilkins, 2004; Lopez *et al*., 2008; Ogendo *et al*., 2008 and Rajendran and Sriranjini, 2008). However, few works has been done in Bangladesh to determine the efficacy of our locally available plant materials against *Tribolium castaneum*. So an investigation was undertaken to determine the residual effects of the stem bark of *Manilkara zapota* (Family: Sapotaceae) on the red flour beetle, *Tribolium castaneum*.

Material and methods

Plant material. Stem bark of *Manilkara zapota* was collected in the month of October, 2010 from Rajshahi district of Bangladesh. The plant material was taxonomically identified by Professor A.T.M Naderuzzaman, Department of Botany, University of Rajshahi and a voucher specimen was deposited under the accession number DACB-23801 at the Bangladesh National Herbarium.

Extraction and TLC screening. The collected stem bark was cleaned and shade-dried. The dried stem bark was then pulverized into a coarse powder by a grinding machine (FFC-15, China). The powdered stem bark (290 gm) was extracted with ethyl acetate at room temperature. The extract was then filtered through filter papers and filtrate was evaporated under reduced pressure at 40°C using a rotary evaporator to have 3.5 g ethyl acetate extract of stem bark. The ethyl acetate extract was run on pre-coated silica gel plate using n-hexane and ethyl acetate (9:1) as the mobile phase and vanillin-H_2SO_4 reagent was used as spray reagent. Ethyl acetate extract of stem bark gave positive test for steroids, glycosides, terpenoids and flavonoids (Harbone, 1984)

Insects. Red flour beetle *Tribolium castaneum* was used to examine the pesticidal activity of ethyl acetate extract of stem bark of *Manilkara zapota*. Adult and larval stages of insect were taken from the Department of Zoology, University of Rajshahi, where pest culture has been maintained for last 10 years in an incubator at 30±1°C, 65% relative humidity and 12:12 hrs dark/light photoperiod, which has been reported as an optimum for rapid growth (Saleem and Shakoori, 1986). Insects were reared on a diet mixture of whole meal flour with Bakers yeast (19:1) in a Jar (Mondal, 1992). After every three days the medium was replaced by a fresh one to avoid conditioning by the larvae (Mondal, 1983).

Residual film method of toxicity. Residual film method as described by Busvine (1971), was used. A preliminary screening of different doses was performed on several instars of larvae and adults to obtain 0% to 100% mortalities. Then 200 mg, 100 mg, 50 mg and 25 mg of ethyl acetate extract of *Manilkara zapota*, was dissolved separately in 5 ml of corresponding solvent to get concentrations of 40 mg/ml, 20 mg/ml, 10 mg/ml and 5 mg/ml, respectively, which were used as stock solutions. 1 ml of various concentrations for each sample was applied on Petri dishes (7 cm diameter) in such a way that it made a uniform film over the Petri dishes. For solvent evaporation, the petridishes were air dried leaving the extract on it. The actual extract present in 1 ml mixture was calculated and the dose per square centimeter was determined by dividing the value present in one ml with the area of the Petri dish. Calculated doses were 1238.5, 619.25, 309.6 and 154.8 μg/cm^2. After drying, 10 beetles were released in each Petri dish with three replications. A control batch was also maintained with the same number of insects after preparing the Petri dish by applying and evaporating the solvent only. The treated beetles were placed in an incubator at the same temperature as reared in stock cultures and the mortality of the beetles was counted after 24 hour post-exposure (Islam *et al*., 2004).

Statistical Analysis. The mortality data was subjected to Probit analysis (Finney, 1971) for the determination of LD_{50} values using the computer software SPSS of 14 version. Results with $p<0.05$ were considered to be statistically significant.

Results

In the present investigation, the toxicity of stem bark of *Manilkara zapota* was tested against both larvae and adults of *Tribolium castaneum*. The mortality (%) was recorded and statistical data regarding LD_{50}, 95% confidence limit and chi-square values were calculated and presented in Table 1. After 72 hrs of exposure, the lowest LD_{50} value of ethyl acetate extract of stem bark of *Manilkara zapota* against 1st, 2nd, 3rd, 4th, 5th, 6th instars larvae and adult *Tribolium castaneum* were found to be 228.8, 281.1, 413.1, 413.4, 423.7, 455.2, 498.7 and 526.5 μg/cm^2, respectively. No mortality was observed in control. The extracts caused

Table 1. Insecticidal activity of stem bark of *Manilkara zapota* against *Tribolium castaneum* (Herbst)

Sample	Life stage	Exposure time (hrs)	LD_{50} (μg/cm^2)	95% Confidence Limits Lower	95% Confidence Limits Upper	Chi-squre (χ^2)
Ethyl acetate extract	1st instar	24	382.9	298.4	491.4	0.275
		48	309.3	247.7	386.2	0.601
		72	228.8	162.7	321.7	0.275
	2nd instar	24	486.3	359.0	658.7	0.106
		48	379.5	303.3	474.9	0.873
		72	281.1	221.5	356.8	0.727
	3rd instar	24	611.5	486.8	768.3	0.075
		48	544.5	425.3	697.0	0.228
		72	413.4	324.2	527.1	5.682
	4th instar	24	636.3	433.4	934.0	1.267
		48	573.6	445.0	739.4	1.041
		72	423.7	334.9	535.9	0.552
	5th instar	24	654.4	468.3	914.7	0.102
		48	580.4	440.9	763.9	1.779
		72	455.2	367.1	564.4	0.219
	6th instar	24	907.4	631.3	1304.3	0.246
		48	751.0	528.0	1068.2	0.080
		72	498.7	407.2	610.8	2.975
	Adult	24	700.6	472.1	1039.6	3.045
		48	652.9	481.5	885.4	0.116
		72	526.5	373.0	742.9	1.626

Values were based on four doses with 30 insects each. # Control groups showed no mortality. *Significant at P<0.05 level.

the highest mortality of the 1st instars larvae in comparison with other larval instars which indicated that the newly hatched larvae were the most susceptible with lowest LD_{50} value (228.8 μg/cm^2) whereas the adults (after 72 hrs exposure) were less susceptible with highest LD_{50} values (526.5 μg/cm^2).

The present result of the experiment first time indicates that like other plant oils extracts, stem bark of *Manilkara zapota* may be used in the control of *Tribolium castaneum* population with integrated pest management system that seems to be economically feasible and ecologically sound.

Discussion

For management of insect pest, many plant products such as essential oils and solvent extracts have been screened for their repellent, toxic and growth inhibitory activities against stored grain pests (Matthews, 1993). Results of this study demonstrated that toxicity of the plant extracts decreased with the increase of age of the larvae. This may clearly support of others that insect's age play an important role in influencing susceptibility (Muwangi and Mukiama, 1988). The present result is more or less similar to the findings of Mondal (1994) and Talukder (1995) who reported the insecticidal properties of neem oil, Pithraj (*Aphanamixis polystachya)* seed extracts against *Tribolium* beetles and also similar to the findings of Upadhyay (2007) who revealed the insecticidal properties of *Piper nigrum* against *Tribolium castaneum*.

Developing countries in Asia and Africa including Bangladesh, have a long history of protection of stored grains with locally available herbal substances, where the application of plant materials is simple and aqueous extracts in several cases proved to be highly effective against stored product insects (Mondal, 1994). Moreover, the crude extracts, oil, leaf powder etc. are easy to prepare and handle and very much cheaper in comparison to the imported chemical pesticides (Yang *et al.*, 2004). Therefore, the use of plant products (crude extracts, oil, powders etc.) as insecticides in stored product protection might benefit the farmers by a reduction of protection costs, insecticide resistance development and environmental impact in term of insecticidal hazard.

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