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Evaluation of insecticidal activity of *Lawsonia inermis* Linn. against the red flour beetle, *Tribolium castaneum* (Herbst)

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ABSTRACT

Lawsonia inermis Linn. (Lythraceae), commonly known as *Henna*, is a popular skin and hair coloring agent in many parts of the world. It is traditionally used as a medicinal plant by diverse groups of tribal/ethnic people. The present work was aimed at studying the insecticidal activity of leaf and fruit of *Lawsonia inermis* against the red flour beetle, *Tribolium castaneum* (Herbst) which is considered as a major pest of stored grains. The leaves and fruits of *Lawsonia inermis* were successively extracted with ethyl acetate and methanol at room temperature to yield EAL (ethyl acetate extract of leaves), EAF (ethyl acetate extract of fruits), MEL (methanol extract of leaves) and MEF (methanol extract of fruits). Then residual film toxicity method was applied to evaluate the insecticidal activity of EAL, EAF, MEL and MEF against adult *Tribolium castaneum*. In this study, the effectiveness of test samples was increased with the increase of exposure time and after 48 hrs of exposure, the lowest LD_{50} was found to be 0.105 mg/cm^2 for MEL. According to the intensity of activity, the toxicity was found in the order of MEL > EAL > EAF > MEF. The overall results of this study suggest that leaves of *Lawsonia inermis* has potential insecticidal effect which might be used in pest control.

KEYWORDS

Insecticidal;
Lawsonia inermis;
Leaves,
Fruits,
Tribolium castaneum.

INTRODUCTION

Losses due to insect infestation are the most serious threat in grain storage, particularly in developing countries, where poor sanitation and inappropriate storage facilities encourage insect attack[1]. The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae), is a serious insect of stored cereals in tropical and subtropical regions of the world. It is generally found in granaries, mills and warehouses, feeding on wheat flour, rice flour, and other processed cereals. The presence of this red flour beetle in stored foods directly affects both the quantity and quality of the commodity[2]. The insects are controlled by different synthetic chemicals which are harmful for other living organism in same environment. Most of these toxic chemicals enter into the food chain and cause pollution of the environment[3]. So scientists and researchers are in continues struggle to make the environment comfortable

and friendly by introducing biodegradable materials for insects control. To minimize the use of synthetic insecticides and to avoid environmental pollution, natural substances like plant extracts have been searched for insect control in recent times. As a result of this type of searching, insecticidal activity of many plants against several insects has been demonstrated[4-5]. As a continuation of this type of searching, we have selected *Lawsonia inermis* L., (commonly called as Henna), which is a well known medicinal plant in Indian subcontinent including Bangladesh. *Lawsonia inermis* is a glabrous, much branched shrub or small tree[6]. Its leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments such as rheumatoid arthritis, headache, ulcers, diarrheoa, leprosy, fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective etc[7]. Moreover, the main constituents reported on this plant are naphthoquinone derivatives, aliphatic components, triterpenes, sterols, phenolic derivatives, coumarins, xanthones, and flavonoids[8]. But there is no report on the insecticidal activity of leaf and fruit of *Lawsonia inermis*. Based on this fact, the objective of the present study is to explore the insecticidal activity of the methanol and ethyl acetate extracts of the leaf and fruit of *Lawsonia inermis* against adult *Tribolium castaneum*.

EXPERIMENTAL

Plant material

Leaves and fruits of *Lawsonia inermis* (Family: Lythraceae) were collected from relevant area of Rajshahi district, Bangladesh. The plant materials were taxonomically identified by Professor A.T.M Naderuzzaman, Department of Botany, University of Rajshahi and a voucher specimen was deposited at the Bangladesh National Herbarium.

Preparation of extracts

The collected leaves and fruits were cleaned and shade-dried. The dried plant materials were then pulverized into a coarse powder. Then the powdered leaves were successively extracted with ethyl acetate and methanol at room temperature. These two extracts were evaporated under reduced pressure at 40°C using a rotary evaporator to have ethyl acetate (EAL) and methanol (MEL) extracts of leaves of *Lawsonia inermis*. Following similar procedure, we found ethyl acetate (EAF) and methanol (MEF) extracts of powdered fruits of *Lawsonia inermis* were obtained.

Insects

We have examined the insecticidal activity of leaf and fruit of *Lawsonia inermis* against Red flour beetle *Tribolium castaneum*. Adult *Tribolium castaneum* were taken from the Department of Zoology, University of Rajshahi, where this insects were cultured in an incubator maintaining optimum conditions (30±1°C, 65% relative humidity and 12:12hr. dark/light photoperiod) for rapid growth[9]. Insects were reared on a diet mixture of whole meal flour with Bakers yeast (19:1) in a Jar[10]. After every three days the medium was replaced by a fresh one to avoid conditioning by the larvae[11].

Residual film method of toxicity

Residual film method as described by Busvine, was used[12]. A preliminary screening of different doses was performed on adult *Tribolium castaneum* to obtain 0% to 100% mortalities. Then 400 mg, 200 mg, 100 mg, 50 mg and 25 mg of each test sample (EAL, MEL, EAF and MEF) were dissolved separately in 5 ml of corresponding solvent to get concentrations of 80 mg/ml, 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 petridishes (7 cm diameter) in such a way that it made a uniform film over the petridishes. For solvent evaporation, the petridishes were air dried leaving the extract on it. The actual extract present in 1ml mixture was calculated and the dose per square centimeter was determined by dividing the value present in one ml with the area of the petridish. So calculated doses were 2.079 mg/cm^2, 1.040 mg/cm^2, 0.520 mg/cm^2, 0.260 mg/cm^2 and 0.130 mg/cm^2. After drying 10 beetles were released in each petridish with three replication. A control batch was also maintained with the same number of insects after preparing the petridish by applying and evaporating the solvent

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only. Insect mortality was recorded 12 hrs, 24 hrs, 36 hrs and 48 hrs after treatments[13].

Statistical analysis

The mortality data were subjected to Probit analysis[14] 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 AND DISCUSSION

In this study, the toxicity of leaf and fruit of *Lawsonia inermis* was tested against adults *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. The LD_{50} values of EAL, MEL, EAF and MEF at 24 hours after treatment were 2.133, 1.755, 4.397 and 5.022 mg/cm^2, respectively whereas at 36 hours after treatment, LD_{50} values were 1.365, 0.325, 1.365 and 4.501 mg/cm^2, respectively. After 48 hrs of exposure, the lowest LD_{50} values for EAL, MEL, EAF and MEF were found to be 0.943, 0.105, 0.927 and 3.049 mg/cm^2, respectively against adult *Tribolium castaneum*. No mortality was observed in control.

There is a renewed interest amongst scientists to study the bioactivity of plant extracts against stored-grain insect pests[15-16]. In this study, methanolic extract of leaf of *Lawsonia inermis* was highly effective in respect to other samples. The order of toxicity of the four different extracts on red flour beetle, *T. castaneum* were: MEL > EAL > EAF > MEF (TABLE 1). The mortality percentage was directly proportional to the level of concentration of plant extract. Moreover, the mortality caused by each sample was increased with the increasing of exposure time. This may clearly support of others that exposure time play an important role in influencing susceptibility[17]. The results of this study are in conformity with the results of Mamun et al[18] who reported that *T. castaneum* adults were significantly more susceptible to the toxicity of the hexane and water extract of *Azadirachta indica*.

The secondary metabolites of plants are vast repository of compounds with wide range of biological activity. It has been reported that the steroids, phenolic compounds and tannins had great impact on insecticidal acitivities. Phytochemical investigation on leaf and fruit of *Lawsonia inermis* have shown the presence of naphthoquinone derivatives, aliphatic components, triterpenes, sterols, phenolic derivatives, coumarins, xanthones and flavonoids[8]. So, the potent activity of MEL against *T. castaneum* may be due to the presence of different classes of bioactive compounds.

The overall results of this study reported the toxic

TABLE 1 : Insecticidal activity of stem bark of *Manilkara zapota* against *Tribolium castaneum* (Herbst)

Sample	Exposure time (hrs)	LD_{50} (mg/cm^2)	95% Confidence Limits		Chi-squre (χ^2)
			Lower	Upper	
EAL	24	2.133	1.276	3.567	1.170
	36	1.365	0.924	2.015	0.930
	48	0.943	0.628	1.226	1.260
MEL	12	2.454	1.323	4.550	1.100
	24	1.755	0.887	3.471	7.464
	36	0.325	0.252	0.420	3.725
	48	0.105	6.984	0.157	1.643
EAF	24	4.397	1.856	10.41	1.175
	36	1.365	0.993	11.51	3.616
	48	0.927	0.579	1.484	3.328
MEF	24	5.022	1.910	2.801	2.742
	36	4.501	0.469	0.965	0.913
	48	3.049	0.307	1.215	1.271

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

effect of leaf of *Lawsonia inermis* on *T. castaneum* and our next approach will be targeted to isolate possible active compounds.

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