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Research Article

Insecticidal activity of plant extracts against Tribolium castaneum Herbst

M. Mostafa*, Hemayet Hossain, M Anwar Hossain, Pizush Kanti Biswas, M. Zahurul Haque

Chemical Research Division, BCSIR Laboratories, Dhaka- 1205, Bangladesh

**Corresponding author: mostafabcsir@yahoo.com*

ABSTRACT

The insecticidal activity of n-hexane, methanol and water extracts of *Tamarindus indica*, *Azadirachta indica*, *Cucumis sativus*, *Eucalyptus species*, *Switenia mahagoni*, and *Psidium guajava* leaves were investigated by using the Film residue method against a red flour beetle Tribolium castaneum Herbst. The results showed that four plant extracts showed a strong to moderate toxicity at a different concentration on red flour beetle. Among them, *Cucumus sativus* leaves extract showed highest mortality (80%) whereas *Psidium guajava* extract showed lowest mortality (50%). Among the solvents, the hexane extracts showed more toxic effect than other extracts. The LC50 results revealed that the hexane extract of *Cucumus sativus* is the most toxic to the pest followed by the hexane extracts of *Azadirachta indica* and *Tamarindus indica*. Qualitative phytochemical analysis has also been performed.

Keywords: *Azadirachta indica*, *Tamarindus indica, Cucumis sativus, Eucalyptus species, Switenia mahagoni, Psidium guajava,* insecticidal activity

1. INTRODUCTION

The protection of stored grain and seeds against insect pests has been a major problem from the development of agriculture. Plant products have been successfully exploited as insecticides, insect repellents and insect antifeedants [1-3] Higher plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control [4]. Insecticidal activity of many plants against several insect pests has been demonstrated [5-7]. 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 eduction of fecundity and fertility. Yang and Tang [8] reviewed the plants used for pest insect control and found that there is a strong connection between medicinal and pesticidal plants.

To minimize 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 [9-11]. However, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and environmentally friendly. Considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides. The aim of our study is to evaluate the insecticidal activity of the hexane, methanol and water extracts of *Tamarindus indica*, *Azadirachta indica*, *Cucumis sativus*n, *Lens culinaris*, *Eucalyptus species*, *Switenia mahagoni*, and *Psidium guajava leaves*

2. MATERIAL AND METHODS

2.1. Plant materials

The *Tamarindus indica* leaves (Tentul), *Azadirachta indica* leaves (Neem), *Cucumis sativus* leaves (Shasha) and *Lens culinaris* (Masur) were collected from the Norshindi district, Bangladesh. The *Eucalyptus species* (Eucalyptus) and *Switenia mahagoni* (Mahogoni) were collected from the BCSIR campus, Dhaka and the *Psidium guajava* leaves (Goam) was collected from the BCSIR Laboratories, Rajshahi campus. The leaves were dried under shade and finally dried in an oven at 45^0C for 48 hours before grinding. The dried plant materials were ground into powder with an electrical blender.

2.2. Extraction of plant materials

The plant powders (100 g each plant sample) were separately extracted in hexane, methanol and water for 24 h on an orbital shaker. The extracts were filtered using a Buchner funnel and Whatman no. 1 filter paper. The hexane, methanol extracts were evaporated to dryness under reduced pressure at 40^0C using a vacuum rotary evaporator, while the water extract was freeze-dried with Savant Refrigerated Vapor Trap. Each extracts were kept in freeze for further work in future.

2.3. Insect bioassays

2.3.1. Test Insects

The red flour beetle, *T. castaneum* were collected from the stock cultures maintained in the BCSIR Laboratories, Rajshahi. Mass cultures were maintained in glass jars (1000ml) and subcultures were in beakers (500ml) with food medium and kept in an incubator at 30±0.5°C. A standard mixture of whole-wheat flour with powdered dry yeast in a ratio of 19:1 [12, 13] was used as food medium throughout the experimental period.

2.4. Mortality tests

Film residue method [14] was used to test the mortality of the adults of *T. castaneum*. The extracted materials were weighed and dissolved in acetone for dosing. For testing beetle, mortality four doses were used including control (water). Ten to fifteen day-old adults of *T. castaneum* was used at 372.95, 785.91 and 1571.83 μg/cm^2 concentrations. The doses were prepared by mixing the requisite quantities of the product with 1 ml acetone/ water. After mixing properly the liquid was dropped in a petri dish (9.5-cm diameter). After drying by fanning and finally in an oven at 40 ºC, 20 adults of each species were released in each Petri dish. For each dose three replications were taken. The doses were calculated by measuring the weight of prepared product (μg) in 01 ml of water divided by the surface area of the petri dish and it was converted into $\mu g/\text{cm}^2$. Mortality was assessed after 24, 48 and 72 h of the treatment. The calculation of mortality rate was corrected for control mortality according to Abbott's formula [15]:

$$Mc = (Mo–Mc/100–Me) \times 100$$

Where, Mo = Observed mortality rate of treated adults (%), Me = mortality rate of control (%), and Mc = corrected mortality rate (%)

The LD_{50} values were determined by probit analysis [14]. The experiments were performed in the laboratory at 30ºC ± 0.5ºC.

2.5. Statistical analyses

The experiment results were statistically analyzed by the mean of one-way analysis of variance ANOVA and when results were significant at p =0.5, Ducan test was used.

2.6. Phytochemical screening

Phytochemical screening of the extracts was carried out using the standard procedures described by Edeoga et al [16] for alkaloids, saponins, tannins, flavonoids, anthraquinones and steroids.

3. RESULTS AND DISCUSSION

The results of preliminary phytochemical analysis of various extracts of *Tamarindus indica*, *Azadirachta indica*, *Cucumis sativus*, *Eucalyptus species*, *Switenia mahagoni* and *Psidium guajava* are presented in Table 4. The hexane extracts of *Azadirachta indica*, *Cucumis sativus* and *Tamarindus indica* showed the presence of steroids and saponins. The methanol extracts of *Azadirachta indica*, *Eucalyptus species*, *Psidium guajava, Switenia mahagoni* and *Tamarindus indica* showed the presence of flavonoids, tannins and saponins, whereas the water extracts of *Azadirachta indica*, *Cucumis sativus*, *Psidium guajava, Switenia mahagoni* and *Tamarindus indica* contained flavonoids and tannins.

The toxic effects of hexane, methanol and water extracts of *Tamarindus indica*, *Azadirachta indica*, *Cucumis sativus*, *Eucalyptus species*, *Switenia mahagoni* and *Psidium guajava* were evaluated against red flour beetle, *T. castaneum* by using the method of residual film technique. Six different extracts of *Tamarindus indica* (TI-1), *Azadirachta indica* (AI-1 & AI-3), *Cucumis sativus* (CS-1 & CS-2) and *Psidium guajava* (PG-1) at different concentration revealed toxicity but any solvent extracts of two plants, *Eucalyptus species* and *Switenia mahagoni* did not showed any toxic effect to red flour beetle. The numbers of dead red flour beetle were counted after 24, 48 and 72 hours at all doses 198.48, 392.95, 785.91 and 1571.83 μg/cm^2 respectively. Then the percentages of corrected mortality were calculated by using Abbott's formula and the results are shown in Table-1. The results showed that the hexane extract of *Cucumis sativus* (CS-1) possessed the highest toxicity at all doses but the hexane extracts of *Azadirachta indica* (AI-1), *Tamarindus indica* (TI-1) and the methanol extract of *Cucumis sativus* (CS-2) showed the moderate toxicity at concentrations 785.91 and 1571.83 μg/cm^2, whereas the hexane extract of *Psidium guajava* leaf (PG-1) possessed the lowest (50%) toxic effect at highest concentration1571.83 μg/cm^2 against red flour beetle, *T. castaneum*. The order of toxicity of the six different plant extracts on red flour beetle, *T. castaneum* were: *Cucumis sativus* (CS-1) > *Azadirachta indica* (AI-1) > *Cucumis sativus* (CS-2) > *Tamarindus indica* (TI-1) > *Azadirachta indica* (AI-3) > *Psidium guajava* (PG-1). The mortality percentage was directly proportional to the level of concentration of plant extract.

The results of the probit analysis for the estimation of LC_{50} values, 95% confidence limits and regression equation at 24, 48 and 72h for the mortality of red flour beetle are presented in Table 2. The LC_{50} values of hexane extracts of *Cucumis sativus* (CS-1*), Azadirachta indica* (AI-1), *Tamarindus indica* (TI-1) and *Psidium guajava* (PG-1) at 24 hours after treatment are 20.64, 234.57, 732.53 and 1944.40 μg/cm^2, at 48 hours after treatment are 24.43, 91.80, 178.74 and 1944.40 μg/cm^2 and at 78 hours after treatment are 10.74, 155.13, 58.36 and 774.22 μg/cm^2 respectively. The LC_{50} values of methanol extract of *Cucumis sativus* (CS-2) at 24 hours after treatment is 557.87 μg/cm^2, at 48 hours after treatment is 153.32 μg/cm^2 and at 78 hours after treatment is 20.64 μg/cm^2 respectively.

The LC_{50} values of water extract of *Azadirachta indica* (AI-3) at 24 hours after treatment is 990.26 μg/cm^2, at 48 hours after treatment is 38.02 μg/cm^2 and at 78 hours after treatment is 38.02 μg/cm^2 respectively. The results indicated that the hexane extract of *Cucumis sativus* (CS-1) at 72 hours after treatment was the most toxic (10.74μg/cm^2) and the hexane extract of *Psidium guajava* (PG-1) was the least toxic (1944μg/cm^2). The hexane extract of *Cucumis sativus* (CS-1) also maintained its toxicity, when the LC_{50} values were compared at 24 HAT (20.64%) and 48 HAT (24.43%). The Chi-square values of different plant extracts at different HAT were insignificant at 5% level of probability and did not show any heterogeneity of the mortality data. The present study results are in conformity with the results of Mamun et al [17] who reported that *T. castaneum* adults were significantly more susceptible to the toxicity of the hexane and water extract of *Azadirachta indica*. Khalequzzaman and Sultana [18] also reported the toxic effect of petroleum ether extract of *Annona squamosa* seed on *T. castaneum*. The toxic and sterilizing effects of *A. calamus* rhizome oil to certain stored grain insects have also been reported by Saxena and Mathur [19]. 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. The different plants extracts in our present study revealed the toxicity against store insect may be due to the presence of different classes of bioactive compounds.

Table 1: Mortality percentage of red flour beetle, T. castaneum treated with different plant extracts by Film residue method

Name of the Plants	Concentration (μg/cm^2)	No of Insect used	No of Insect dead			Total No of Insects dead	% of Average Mortality	% Corrected Mortality
			24 hrs	48 hrs	72 hrs			
CS1	196.48	120	84	90	96	90	75	75
	392.95		90	90	96	92	76.66	76.66
	785.91		96	96	96	96	80	80
	1571.83		96	96	96	96	80	80
	Control		0	0	0	0		
AI1	196.48	120	60	66	66	64	53.33	53.33
	392.95		60	90	72	74	61.66	61.66
	785.91		78	90	90	86	71.66	71.66
	1571.83		78	96	96	90	75	75
	Control					0		
TI1	196.48	120	30	66	72	56	46.66	46.66
	392.95		42	66	90	66	55	55
	785.91		72	90	96	86	71.66	71.66
	1571.83		72	96	96	88	73.33	73.33
	Control					0		
PG1	196.48	120	30	30	30	30	25	25
	392.95		30	30	36	32	26.66	26.66
	785.91		54	54	72	60	50	50
	1571.83		54	54	72	60	50	50
	Control					0		
CS2	196.48	120	30	66	84	60	50	50
	392.95		66	72	90	76	63.33	63.33
	785.91		66	96	96	86	71.66	71.66
	1571.83		78	96	96	90	75	75
	Control		0	0	0	0		
AI3	196.48	120	42	72	72	62	51.66	51.66
	392.95		60	84	84	76	63.33	63.33
	785.91		60	84	84	76	63.33	63.33
	1571.83		60	90	90	80	66.66	66.66
	Control		0	0	0	0		

Table 2. χ2-values, regression equations, LD_{50} and 95% confidence limits of some indigenous medicinal plant extracts against T. castaneum, a stored grain insect pests after 24, 48 and 72 h of treatment

Plant extracts	Hrs after treatment	Test insect	χ^2- values for Heterogeneit*	Regression equations	LD_{50} ($\mu g.cm^{-2}$)	95% Confidence limits	
						Lower	Upper
CS 1	24 h	*T. castaneum*	0.810	Y= 4.413+0.465X	20.64	0.00	89.05
	48 h	*T. castaneum*	1.713	Y= 4.305+0.492X	24.43	0.00	97.72
	72 h	*T. castaneum*	0.877	Y= 4.34+0.64X	10.74	0.00	77.92
AI1	24 h	*T. castaneum*	2.205	Y= 3.80+0.505X	234.578	36.99	405.10
	48 h	*T. castaneum*	4.111	Y= 3.64+0.708X	91.807	16.51	174.99
	72 h	*T. castaneum*	1.093	Y= 1.70+1.57X	155.13	59.75	242.74
TI1	24 h	*T. castaneum*	5.195	Y= 0.73+1.56X	732.538	579.51	974.10
	48 h	*T. castaneum*	3.734	Y= 3.01+0.89X	178.741	80.14	267.11
	72 h	*T. castaneum*	2.886	Y= 3.88+0.64X	58.368	3.95	135.43
PG1	24 h	*T. castaneum*	4.222	Y= 2.63+0.718X	1944.40	1182.78	6493.45
	48 h	*T. castaneum*	4.222	Y= 2.63+0.718X	1944.40	1182.78	6493.45
	72 h	*T. castaneum*	7.599	Y= 1.61+1.17X	774.22	617.66	1025.98
CS2	24 h	*T. castaneum*	9.380	Y= 2.12+1.05X	557.87	425.44	730.78
	48 h	*T. castaneum*	3.683	Y= 3.03+0.90X	153.32	64.66	234.95
	72 h	*T. castaneum*	0.810	Y= 4.413+0.465X	20.64	0.00	89.05
AI3	24 h	*T. castaneum*	3.352	Y= 3.87+0.38X	990.26	495.08	126631.03
	48 h	*T. castaneum*	0.874	Y= 4.34+0.42X	38.02	0.00	137.92
	72 h	*T. castaneum*	0.874	Y= 4.34+0.42X	38.02	0.00	137.92

**χ2 = Goodness of fit. The tabulated value of χ2 is 5.99 (df = 2, P<0.05)*

Table 3: Results of phytochemical Tests

Plant name	Extract	Alkaloid	Steroid	Flavonoid	Tannins	Saponins
AI	Hexane	-	+	-	-	-
	Methanol	-	-	+	++	-
	Water	-	-	++	++	-
CS	Hexane	-	++	-	-	+
	Methanol	-	-	-	-	-
	Water	-	-	+	++	-
ES	Hexane	-	-	-	-	-
	Methanol	-	-	++	++	+
	Water	-	-	-	-	-
PG	Hexane	-	-	-	-	-
	Methanol	-	-	-	++	-
	Water	-	-	-	-	-
SM	Hexane	-	-	-	-	+
	Methanol	-	-	++	+++	+
	Water	-	-	-	-	++
TI	Hexane	-	+	-	-	-
	Methanol	-	-	+	-	++
	Water	-	-	++	+	-

+ = presence, -= Absence

From the insecticidal activity results, it is observed that different solvent extracts of four plants would be more or less effective for controlling red flour beetle. The hexane extract of *Cucumis sativus* showed the highest toxic effect followed by the hexane extract of *Azadirachta indica* . *Cucumis sativus* is available throughout the country and the farmers may use this plant in their storehouses for the management of stored grain pests.

Further investigation on the identification of active ingredient from the hexane extracts, which is more effective than other extracts, is utmost needed.

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