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Insecticidal Activity of Flower of *Tagetes erecta* L. against *Tribolium castaneum* (Herbst)

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Abstract: The crude extracts and its fractions of the flower of *Tagetes erecta* Linn. were tested for insecticidal activity against a stored product insect pest, *Tribolium castaneum* (Herbst). The chloroform fraction showed highest toxicity against both the larvae and adults of *Tribolium castaneum* followed by petroleum ether fraction and ethanol extract. The LC_{50} values of chloroform fraction against first, second, third, fourth, fifth and sixth instar larvae were 11.64, 14.23, 19.26, 29.02, 36.66, 59.51 $\mu g/cm^2$ (72 h.), respectively and for adults the value was 65.93 $\mu g/cm^2$ (72 h.). No mortality was observed in control. Finally we can conclude that the flower of *Tagetes erecta* might be a pesticide against *Tribolium castaneum*.

Key words: Insecticidal, *Tagetes erecta*, *Tribolium castaneum*

INTRODUCTION

The red flower beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a common and most destructive pest throughout the world[1]. It is generally found in granaries, mills, warehouses and live on cracked grain on breakfast food or meal, rice, dried fruit, bleached and unbleached wheat flour, cornmeal, barley flour and oatmeal[2]. This pest has also 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[3]. In tropical countries like Bangladesh, the climate and storage conditions are favorable for insect growth and development[4]. More than 2000 species of field and storage pests annually destroy approximately one third of world's food production, valued US $ 100 billion among which highest losses (43% of potential production) occur in developing Asian countries[5].

Use of chemical pesticides is the easiest solution. But the indiscriminate use of chemical pesticides has given rise to many serious problems, including genetic resistance of pest species, toxic residues, increasing costs of application, environmental pollution and hazards from handling etc[6-7]. 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[8-13]. There is an urgent need for safe but effective, biodegradable pesticides with no toxic effects on organisms. Botanical insecticides are broad-spectrum in pest control and many are safe to apply, unique in action and can be easily processed and used. Locally available plants and minerals have been widely used in the past to protect stored products against damage by insect infection[14]. The main advantage of botanicals is that they are easily produced by farmers, small-scale industries and are potentially less expensive.

The present experiment was undertaken to establish the action of a plant, *Tagetes erecta* Linn. on the rust-red flour beetle, *Tribolium castaneum* (Herbst). This plant is locally known as "Genda Phul" (English-Marigold) belongs to the family Compositae (Asteraceae) and is widely distributed in South East Asia including Bangladesh and India[15]. Different parts of this plant including flower are used in folk medicine to cure various diseases like fevers, epileptic fits (Ayurveda), astringent, carminative, stomachic, scabies and liver complaints. The flowers are also employed in diseases of the eyes and for ulcers. Internally, they are said to purify blood and flower juice is given as a remedy for bleeding piles[15-16]. In our laboratory we have also found the antibacterial, antifungal and cytotoxic effects (against brine shrimp nauplii) of the flowers of *Tagetes erecta*[17]. These lead us to evaluate the effects of ethanol extract and its different fractions of the flower of *Tagetes erecta* Linn. against the different larval and adult stages of *Tribolium castaneum* (Herbst).

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MATERIALS AND METHODS

Plant Collection: Fresh flowers of *Tagetes erecta* Linn. were collected from the adjoining areas of Rajshahi University Campus, during the month of December to January and taxonomically identified by Professor A.T.M. Naderuzzaman, Department of Botany, University of Rajshahi, Bangladesh, where a voucher specimen (No. J. Sultana 23, collection date 17.01.1994) has been deposited.

Extraction and Fractionation: The fresh flowers of *Tagetes erecta* were sun dried for 7 days and finally in an electrical oven below 60ºC for 48 hours. The dried plant materials (1kg) were then extracted in room temperature with ethanol (5.0 lit.). The filtrate was concentrated and fractionated with petroleum ether and chloroform. The solvents were evaporated by rotary evaporator at 40ºC under reduced pressure to afford a brownish syrupy suspension of ethanol extract (50.0gm), petroleum ether soluble fraction (18.6 gm) and chloroform soluble fraction (23.8 gm).

TLC Screening: All extracts were run on pre-coated silica gel plate using petroleum ether and ethyl acetate (9:1 and 7:5) as the mobile phase and vanillin-H_2SO_4 reagent was used as spray reagent. Ethanol extract of flower gave positive test for glycosides but the chloroform and petroleum ether soluble fractions mainly showed the presence of terpenoids and flavonoids[18].

Test Insects: All larvae and adults of *Tribolium castaneum* were collected from the Department of Zoology, University of Rajshahi, where pest culture maintained for last 10 years in an incubator at 30±1ºC, 65% relative humidity and 12:12hr. dark/light photoperiod which has been reported an optimum for rapid growth[19]. Insects were reared on a diet mixture of whole meal flour with Bakers yeast (19:1) in a jar[20] at the Biochemistry and Molecular Biology Department, Rajshahi University. Adult beetles of both sexes were collected in the pupal stage and unmated male and female adult aged between 10-20 days were used in the experiment[21]. After every three days the medium was replaced by a fresh one[22] to avoid conditioning by the larvae[23].

Insecticidal Bioassay: The insecticidal activity of crude extracts and its fractions were determined by Residual film method[24]. The crude ethanol extract (350 mg), chloroform fraction (80 mg) and petroleum ether fraction (250 mg) were dissolved in 10 ml of corresponding solvent in order to get a stock solution. Then desired serial dilutions were prepared from the each stock solution using corresponding solvents. Various concentrations of extract/fractions (1ml each) were applied to petridishes (5 cm diameter). The petridishes were air dried leaving the extract/fractions on it. The actual concentrations were calculated by measuring the dry weight of the crude extract/fractions (present in 1 ml) applied into the petridishs divided by the surface area of the respective petridishes. In this experiment, the calculated doses were ranges from 11.32 $\mu g/cm^2$ to 356.68 $\mu g/cm^2$ for different instar larvae and for adult. After evaporation of solvents and drying off the dishes at room temperature, 30 randomly selected larvae and adults were transferred to each dish and left without food for 24, 48 and 72 hours. Control dishes treated as above, but with solvents only. Those insects (larvae and adult) that did not move when prodded gently with a brush were considered as dead. All experiments were conducted separately under laboratory condition at 30ºC.

Statistical Analysis: The mortality data of different instars of larvae and adults were corrected by Abbott.s formula[25]. The median lethal doses LC_{50} values for each inster and adult were calculated by Probit analysis as described by Finney[26].

RESULTS AND DISCUSSION

In this present investigation, the toxicity of *Tagetes erecta* was tested against six different larval and adult stages of *Tribolium castaneum*. The data were recorded and statistical data regarding LC_{50}, 95% confidence limit and Chi-square value were calculated and the results of ethanol extract, petroleum ether fraction and chloroform fraction are presented in Table 1, 2 and 3, respectively. The chloroform fraction (Table 3) of the flower of *Tagetes erecta* was more toxic to both larvae and adults of *Tribolium castaneum* than the ethanol extract (Table 1) and petroleum ether fraction (Table 2) as compared the LC_{50} values. At 72 hour exposure, the LC_{50} values of chloroform fraction against first, second, third, fourth, fifth and sixth instar larvae were 11.64, 14.23, 19.26, 29.02, 36.66, 59.51$\mu g/cm^2$ and for adults was 65.93 $\mu g/cm^2$, respectively (Table 3). In control groups, there was no mortality even without food for 3 days. Results of this study also demonstrated that toxicity of the plant extracts decreased with the increase of age of the larvae. This might be due to relatively small amount of extracts was consumed and quite sufficient to kill them in a large number. The present result is similar to Mondal's[3] report, where eleven plant materials including neem oil exhibited insecticidal effect against *Tribolium* beetles. This result is also similar to the findings of Talukder and Howse[27] and Padin *et al.*[28] who reported the

Table 1: Insecticidal activity of ethanol extract against *Tribolium castaneum* (Herbst).

Sample	Life stage	Exposure time (hrs)	LC_{50} (µg/cm^2)	95% Confidence Limit LEL	95% Confidence Limit UFL	Slope	Chi-square (Degrees of freedom)
Ethanol extract	1st Instar	24	82.18	44.48	151.80	1.00	0.00609 (1)
		48	55.60	31.52	98.07	1.21	0.00911 (1)
		72	31.86	15.64	64.87	1.29	0.40478 (1)
	2nd Instar	24	97.43	52.25	181.67	1.00	0.01926 (1)
		48	69.00	41.39	115.02	1.24	0.06635 (1)
		72	35.57	17.37	72.83	1.22	0.42694 (1)
	3rd Instar	24	121.37	61.78	238.44	0.97	0.00383 (1)
		48	81.51	47.72	139.22	1.17	0.01258 (1)
		72	44.17	23.70	82.30	1.19	0.30393 (1)
	4th Instar	24	177.64	79.20	398.41	0.96	0.02578 (1)
		48	102.19	57.26	182.35	1.09	0.00033 (1)
		72	51.63	26.51	100.54	1.03	0.01524 (1)
	5th Instar	24	269.24	86.04	842.56	0.87	0.01483 (1)
		48	136.52	73.86	252.33	1.12	0.02401 (1)
		72	82.18	44.48	151.80	1.00	0.00609 (1)
	6th Instar	24	385.21	90.83	1633.58	0.82	0.01958 (1)
		48	177.64	79.20	398.41	0.96	0.02578 (1)
		72	116.91	56.19	243.21	0.88	0.04577 (1)
	Adult	24	238.31	148.94	381.31	1.63	0.51587 (1)
		48	180.98	120.97	270.77	1.58	0.44687 (1)
		72	149.34	100.21	222.55	1.55	1.07416 (1)

Values were based on three concentrations with 30 insects each.
Control group (solvent) showed no mortality even after 3 days without food.

Table 2: Insecticidal activity of petroleum ether fraction against *Tribolium castaneum* (Herbst).

Sample	Life stage	Exposure time (hrs)	LC_{50} (µg/cm^2)	95% Confidence Limit LEL	95% Confidence Limit UFL	Slope	Chi-square (Degrees of freedom)
Petroleum ether fraction	1st Instar	24	56.30	29.58	107.15	0.97	0.18318 (1)
		48	35.84	17.32	74.17	1.05	0.05636 (1)
		72	22.27	09.68	51.23	1.20	0.60149 (1)
	2nd Instar	24	87.90	42.28	182.74	0.83	0.23054 (1)
		48	48.60	23.37	101.03	0.89	0.11711 (1)
		72	27.13	10.87	67.72	0.96	0.50201 (1)
	3rd Instar	24	110.34	52.93	230.02	0.91	0.11330 (1)
		48	83.94	38.96	180.83	0.80	0.00318 (1)
		72	33.22	12.95	85.21	0.83	0.69584 (1)

Table 2: Continue

	4th Instar	24	183.17	65.69	510.74	0.86	0.14855 (1)
		48	101.69	49.85	207.44	0.91	0.00011 (1)
		72	53.74	24.76	116.61	0.80	0.19565 (1)
	5th Inster	24	229.96	71.33	741.32	0.87	0.16331 (1)
		48	142.12	63.37	318.69	0.96	0.02577 (1)
		72	87.90	42.28	182.74	0.83	0.23054 (1)
	6th Instar	24	302.96	71.33	863.24	0.74	0.15762 (1)
		48	229.96	65.48	741.32	0.87	0.16331 (1)
		72	142.08	54.87	367.89	0.82	0.16630 (1)
	Adult	24	241.94	167.12	350.26	1.81	0.24355 (1)
		48	195.52	136.23	280.61	1.72	0.39797 (1)
		72	152.13	106.26	217.80	1.77	0.51387 (1)

\# Values were based on three concentrations with 30 insects each.
\# Control group (solvent) showed no mortality even after 3 days without food.

Table 3: Insecticidal activity of chloroform fraction against *Tribolium castaneum* (Herbst).

Sample	Life stage	Exposure time (hrs)	LC_{50} (µg/cm^2)	95% Confidence Limit		Slope	Chi-square (Degrees of freedom)
				LEL	UFL		
Chloroform fraction	1st Instar	24	32.87	17.79	60.72	1.00	0.00614 (1)
		48	19.26	10.11	36.70	1.13	0.00128 (1)
		72	11.64	05.68	23.82	1.32	0.90658 (1)
	2nd Instar	24	38.97	20.90	72.67	1.00	0.01917 (1)
		48	23.25	12.88	41.94	1.12	0.01214 (1)
		72	14.23	06.95	29.13	1.22	0.42704 (1)
	3rd Instar	24	50.93	27.96	92.77	1.11	0.03359 (1)
		48	32.87	17.79	60.72	1.00	0.00614 (1)
		72	19.26	10.11	36.70	1.13	0.00128 (1)
	4th Instar	24	63.82	32.12	126.80	1.07	0.03365 (1)
		48	44.67	23.17	86.08	0.97	0.09490 (1)
		72	29.02	16.56	50.85	1.10	0.00001 (1)
	5th Instar	24	100.18	39.15	256.33	1.00	0.00730 (1)
		48	59.51	28.97	122.23	0.97	0.09856 (1)
		72	36.66	19.71	68.17	0.99	0.01564 (1)
	6th Instar	24	121.91	45.89	323.86	1.09	0.00006 (1)
		48	95.97	33.02	278.93	0.87	0.06337 (1)
		72	59.51	28.97	122.23	0.97	0.09856 (1)
	Adult	24	118.53	76.35	184.03	1.44	0.80873 (1)
		48	89.46	57.17	139.98	1.41	0.69771 (1)
		72	65.93	40.35	107.71	1.62	1.57811 (1)

\# Values were based on three concentrations with 30 insects each.
\# Control group (solvent) showed no mortality even after 3 days without food.

insecticidal properties of Pithraj (*Aphanamixis polystachya)* seed extracts and *Rosmarinus officinalls* oil against *Tribolium castaneum*, respectively. The insecticidal activity of pyrethrin isolated from the callus culture of *Tagetes erecta* against Tribolium spp. was observed[29] but there is no published data on the toxicity of *Tagetes erecta* against *Tribolium castaneum*, to be compared with the present data.

Conclusion: The findings of the present investigation revealed that *Tagetes erecta* has good insecticidal activity and can be used in the control of *Tribolium castaneum* population with integrated pest management system which seems to be economically feasible and ecologically sound. However, more research should be directed towards isolation of bioactive compounds as well as field trials must be conducted before these extracts are used in grain storages.

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