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Pesticidal Evaluation Of *Duranta Repens* Linn. Against *Tribolium Castaneum* (Herbst)

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ABSTRACT

The crude extracts stem and fruit of *Duranta repens* Linn were found to be toxic against both the larvae and adults of a stored product insect pest, *Tribolium castaneum*(Herbst). The chloroform extract of stem showed more toxicity than the ethanol and diethyl ether extracts. At 72 hour exposure the LC_{50} values of chloroform extract for adults was 90.5μg/cm^2 and for first instar larvae was 9.1μg/cm^2, respectively. On the other hand the ethanol extract of fruit extract was found to be more toxic than the chloroform and petroleum ether extracts. The LC_{50} value of ethanol extract for adult was 66.0μg/cm^2 and only 8.3μg/cm^2 for the first instar larvae. In comparison, fruit extract is more potent than the stem extracts and the larvae showed comparative tolerance with the increase of their age and time.

KEYWORDS

Duranta repens Linn;
Tribolium castaneum(Herbst);
Stem;
Fruit;
Toxicity.

INTRODUCTION

Pest control is a major issue for underdeveloped agriculture countries. 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 (Ahmed & Grainge, 1986). 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, hazards from handling etc. (Ahmed et al., 1981; Khanam et al., 1990). 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(Dyte, 1970; Pasalu and Bhatia, 1983; Dyte and Halliday, 1985; Irshad and Gillani, 1990; Zettler and Cuperus, 1990; Zettler, 1991).

There is an urgent need for safe but effective, biodegradable pesticides with no toxic effects on non-target organisms. This has created a world-wide insecticides, and the re-evaluation and use of age-old, traditional botanical pest control agents(Heyde et al., 1983). 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(Golob and Webley, 1980). The main advantage of botanicals is that they are easily produced by farmers and small-scale industries and are potentially less expensive.

The present experiment was undertaken to establish the action of a toxic plant, *Duranta repens* Linn., on the rust-red flour beetle, *Tribolium castaneum* (Herbst.)(Coleoptera:Tenebrionidae). *Tribolium* is a major pest of stored flour and flour-based products in all tropical and subtropical countries of the world(Cotton, 1947; Pruthi and Singh, 1950). Their presence in a stored food results in contamination and substantial economic damage due to loss of the product and a decrease in nutritional value(Wilburt and Mills, 1978).

In several countries locally available plant materials are widely used to protect stored products against insect pests, including *Tribolium* species(Casida, 1976; Islam, 1987; Khanam and Talukder, 1988; Saxeana et al., 1989). It is also reported that eleven plant materials including neem oil exhibited insecticidal effect against *Tribolium* beetles(Mondal, 1994). Seed extracts of Pithraj (*Aphanamixis polystachya)* have showed strong repellent effects and moderate feeding deterrent and insecticidal (direct-contact) effects on adult *T.castaneum*(Talukder and Howse, 1993). Recently, Padin et al. (2000) reported that rosemary (*Rosmarinus officinalls)* oil showed toxicity to *T.castaneum* at a dose of 60mg/L. Similarly, El-Lakwah et al., (1997a) reported the toxic effects of extracts and powders of *D.repens* against the rice weevil(*Sitophilus oryzae* L). They also reported that the plant extracts and powders of *D. repens* were found to be toxic to the lesser grain borer-*Rhizopertha dominica* F.adult(El-Lakwah *et al.*, 1997b). Moreover, El-Naggar and Mosallam(1987) reported that the different solvent extracts of *D. repens* showed antifeedant and insecticidal properties against the larvae of *Culex pipiens* and *Spodoptera littoralis,* and against the adults of *Musca domestica* and *C.pipiens*. But, there is no information on the toxicity of *Duranta repens* against *T.castaneum*.

Primarily in our laboratory, the crude stem and fruit extracts of *D.repens* exhibited the presence of steroids, flavonoids, terpenoids and glycosides on TLC screening. The chloroform extract of stem and ethanol fraction of fruit extract showed mild to moderate *in vitro* antimicrobial activity against both Gram-positive and Gram-negative bacteria. Ethanol fraction of crude fruit extract exhibited comparatively higher toxicity than crude chloroform extract of stem against Brine shrimp nauplii. These lead us to evaluate the toxic effects of fruit and stem extract of *D repens* and its isolates against the larvae and adult stages of *T.castaneum*(Herbst).

MATERIALS AND METHODS

Plant

The plant part(stem and fruits) of *Duranta repens* Linn. (Verbenaceae) was collected from the adjoining areas of Rajshahi University campus during June, 2001 and was identified by Prof.A.T.M. Naderuzzaman, Department of Botany, University of Rajshahi where a voucher specimen number (Alam, 78) has been deposited.

Plant extraction

D.repens stem were sun dried and pulverized into a coarse powder. One kilogram of the ground stem was extracted in cold with ethanol. After concentration, the ethanol extract was fractionated with chloroform and diethyl ether. The solvents were concentrated by rotary evaporator at 40^0C under reduced pressure to afforded a semisolid mass of ethanol, chloroform and diethyl ether extract(90.0, 15.6 and 20.8gm), respectively. Similar extraction process was followed for the 950gm of fruit to obtain a semisolid mass of ethanol, chloroform and petroleum ether extracts(30.0, 8.0 and 6.0gm), respectively.

TLC screening

All extracts were run on pre-coated silica gel plate using hexane and ethyl acetate(2:1 and 1:1) as the mobile phase and vanillin-H_2SO_4 was used as spray reagent. Stem ethanol and diethyl ether extracts gave positive tests for steroids and chloroform extract showed the presence of flavonoids and terpenes. On the other hand, fruit ethanol extract gave positive test for steroids and glycosides and the chloroform and petroleum ether extracts mainly showed the presence of flavonoids(Harbone, 1984).

Isolation

The chloroform soluble fraction(5gm) of stem

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was subjected to a column chromatography eluting with n-hexane and ethyl acetate of increasing polarity which gave a total of 33 fractions. Among these, fractions 8-15 eluted with n-hexane-ethyl acetate(2:1) showed similar spots on TLC and were combined. The combined column chromatographic fraction was then subjected to PTLC using the solvent system n-hexane-ethyl acetate(5:1). The pink colored band was observed in an edge of the chromatogram by spraying with vanillin-H_2SO_4 reagent and was scrapped off and eluted with ethyl acetate and evaporated off under reduced pressure to afford an amorphous powder (mixture) (480mg).

Test insect

All larvae and adults of *T.castaneum* were collected from the Department of Zoology,University of Rajshahi, where pest culture maintained for last 10 years in an incubator at 30±1^{0}C, 65% relative humidity and 12:12hr. dark/light photoperiod which has been reported 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, 1983) 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(Mondal and Aktar, 1992). After every three days the medium was replaced by a fresh one(Mondal, 1984) to avoid conditioning by the larvae(Park, 1934).

Bioassay

Stem ethanol(350mg), chloroform(80mg) and diethyl ether(250mg) and fruit ethanol(40mg), chloroform(60mg) and petroleum ether(70mg) were dissolved in 10ml of corresponding solvent in order to get a stock solution. The isolated amorphous powder mixture(320mg) was also dissolved in 10ml of chloroform. Then desired serial dilutions were prepared from the each stock solution using corresponding solvents. The tests were aimed at establishing complete regress line in order to determine LC_{50} values. Various concentrations(1ml) of each extract (range 10 between 1800μg/cm^2) were applied to petridishes(5cm diameter). 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 30^{0}C.

Statistical analysis

The mortality data of different larvae instars and adults were corrected by Abbott's formula (1925). The median lethal doses LC_{50} for each instar and stage were calculated by probit analysis as described by Finney(1971).

RESULTS AND DISCUSSION

The results of dose-mortality were presented in TABLE 1 which showed that the chloroform extract of the stem of *D.repens* was more toxic to both larvae and adults of *T.castaneum* than the ethanol and diethyl ether extracts. In contrast, ethanol extract of fruit showed better toxicity against both larvae and adults of *T.castaneum*. The isolated amorphous mixture form the chloroform fraction of stem was showed weak toxicity compared to it crude extract. In both control groups, there was no mortality even without food for 3 days. But in both experimental groups, at longer exposure(72 hr.) of the insects on all the extracts resulted in higher mortality rate (Figure 1). The increase in mortality with increase in exposure period could be due to several factors, which may be acting separately or jointly. For example, the uptake of the active moiety of extracts could be time dependent or it could get converted into more toxic metabolites in the larval integument and as well as alimentary canal, regulating in time-dependent effects. TABLE 1 also demonstrated that toxicity of the plant extracts decreased with the increase of age of the larvae. For the stem extracts, the 1st instar larvae were most susceptible to chloroform(9.1μg/cm^2) followed by diethyl ether(61.0 μg/cm^2) and ethanol(77.6μg/cm^2) than the older larval instars. Similarly, for the fruit extracts, the 1st instar larvae were most susceptible to ethanol (8.3μg/cm^2) followed by chloroform (22.5μg/cm^2) and petroleum ether(31.4 μg/cm^2) than the older larval instars. Among the two types of ex-

TABLE 1 : Dose-mortality of crude extracts of *D.repens* against *T.castaneum*.

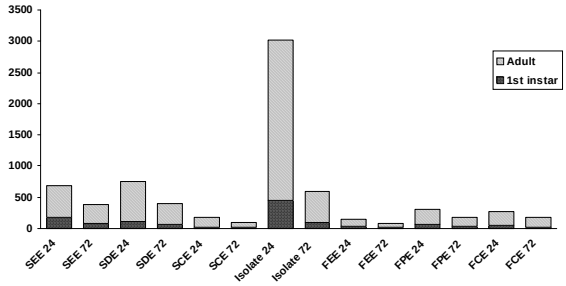
Plant extract	Exposure time(h)	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	6th Instar	Adult (µg/cm²)
				Extracts from the stem				
	24	180.6	289.0	352.4	403.07	432.8	429.1	502.0
Ethanol	48	106.4	257.0	205.0	300.3	305.6	355.6	414.0
	72	77.6	215.2	183.0	206.6	250.9	299.0	313.2
	24	110.5	143.0	346.5	336.8	476.1	543.3	633.5
Diethyl ether	48	79.6	96.4	230.0	244.8	372.9	402.0	448.3
	72	61.0	85.7	129.7	166.8	274.0	332.0	334.2
	24	22.7	32.4	36.6	86.0	117.1	136.1	149.0
Chloroform	48	13.4	19.9	25.4	59.0	61.6	64.2	117.8
	72	9.1	11.5	17.5	43.8	50.5	54.5	90.5
	24	442.5	556.6	1131.0	1131.0	1537.1	1964.1	2579.9
Isolate (mixture)	48	150.5	253.4	352.6	352.5	465.1	486.0	774.1
	72	90.6	107.7	127.3	127.3	170.2	321.5	504.7
				Extracts from the fruit				
	24	26.7	30.2	31.8	38.3	60.0	84.6	118.5
Ethanol	48	19.5	21.5	23.5	27.6	41.1	60.3	89.5
	72	8.3	13.8	17.7	19.4	27.6	38.7	66.0
	24	58.7	66.3	79.6	93.2	140.6	189.5	242.0
Pet. Ether	48	45.1	48.8	63.4	73.9	102.3	140.6	195.5
	72	31.4	34.4	52.8	59.8	75.4	102.3	152.1
	24	40.0	53.5	63.4	92.3	137.3	225.9	238.3
Chloroform	48	29.1	42.0	49.0	65.8	88.8	156.3	181.0
	72	22.5	31.8	36.8	43.0	62.3	95.3	149.3

Values were based on three concentrations with 30 insects each

Control group (solvent) showed no mortality even after 3 days without food

tracts, fruit extracts showed good potency over the stem extracts and the larvae showed comparative tolerance with the increase of their age and time. Figure 1 indicates that after 24 hr. chloroform extract of stem showed more toxicity against 1st instar but in case of adult fruit ethanol extract showed more activity than stem chloroform extract. Figure 1 also demonstrates that stem diethyl ether extract showed lowest activity against both 1st and adult of *T.castaneum*. But in average, all extracts of stem and fruit, were found to more toxic to the 1st larval instar than the adults(Figure 1) and the LC_{50} values for larvae were lower that those of adults(TABLE 1). This might be due to relatively small amount of extracts was consumed and was quite sufficient to kill them in a large number. The weak toxicity of isolate mixture from chloroform was seems to be that they are not responsible for bio-activity and other compounds remained in the crude chloroform extract may exhibited synergistic effect against the tested pest *T.castaneum*.

The present result is similar to Khanam et al., (1990) who reported toxic effect of Royna (*Aphanamixis polystachya)*against *T.confusum*. This result is also similar to the findings of Khalequzzamen et al.(1988) and Jahan et al. (1989) who reported the insecticidal properties of tobaco(*Nicotiana tobactam)* leaf and bishkatali(*Polygonum hydropiper)* against *T.confusum* larvae, respectively. There is no published data on the toxicity of *D. repens* belongs to Verbenaceae, to be compared with the present data. However, another plant from the same family Verbenaceae, *Clerodendron incrme* leaf extract showed dose-dependant repellent and toxicity against adult



SEE=Stem ethanol extract, SDE=stem diethyl ether extract, SCE=Stem chloroform extract, FEE=Fruit ethanol extract, FPE=Fruit petroleum ether extract, FCE= fruit chloroform extract

Figure 1 : Comparison of LC_{50} values of different extracts of stem and fruit against 1st instar larvae and adult of *T.castaneum* after 24 hour and 72 hour

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T.castaneum (Emara and Ryan, 1997).

The present result of the experiment indicates that like other plant oils and extracts, whole plant extracts of *D.repens* may be used in the control of *T.castaneum* population with integrated pest management system which seems to be economically feasible and ecologically sound. This study also confirms the validity of traditional use of Kata mehedi(*D.repens)* against stored grain pests. 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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