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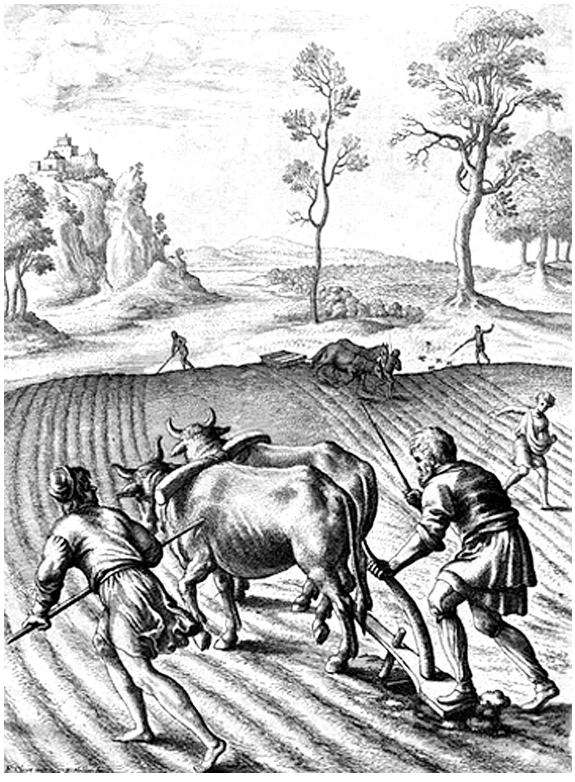

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Pesticidal Activity of a Novel Coumestan Derivative Isolated from *Psoralea corylifolia* Linn. Against *Tribolium casteneum* Herbst. Adults and Larvae (Coleptera:Tenebrionidae)

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Abstract: The pure compound, 6-(-3-methylbut-2-enyl)-6′-7-dihydroxycoumestan (1) isolated from the chloroform extract of the seeds of *Psoralea corylifolia* Linn. was evaluated for the pesticidal activity against both adults and different instars of *Tribolium casteneum* Hebrst. under laboratory conditions. The LD_{50} values for the compound were 910.34, 620.47, 388.45 and 1159.87, 714.88, 404.26 and 1395.70, 740.75, 493.97 and 1605.34, 835.61, 565.83 and 1652.84, 916.79, 729.50 and 1764.32, 994.16, 784.09 and 1678.52, 992.04, 795.67 and 2350.41, 1395.70, 985.12 ppm for the 1st, 2nd, 3rd, 4th, 5th, 6th, adult male and female, respectively at 24, 48 and 72 h post exposure. These results demonstrated that the earlier instars were more sensitive to the compound than those of late instars those follow to those of individual adults.

Key words: Pesticidal activity, novel coumestan derivative, *Psoralea corylifolia* Linn. *Tribolium casteneum* Herbst

Introduction

The red flour beetle, *Tribolium casteneum* is a major pest of stored food grains and cosmopolitan in distribution (Good, 1933). Both adults and larvae are able to exploit a wide variety of stored commodities (Ziegler, 1977). Their presence in the stored product results in both contamination and substantial economic damage due to loss of the products market price and decreases nutritional value as well (Barkholder and Faustini, 1991).

Considerable works has been done on the toxicity of different chemical insecticides used for the protection of *Tribolium casteneum* under different conditions (Tyler and Binns, 1977; Yadav *et al.*, 1983). Synthetic chemical pesticides proved to be unduly toxic to all forms of life, ecologically derastrous, which led to frantic search for safer pest control agents. Recently photochemical pesticides have received much attention for their environmental friendliness. With the invention of synthetic pesticides, research on plant derived pesticides dismissed. The synthetic pesticides, however, have become under increasing attack in recent years due to the persistence in the environment that produce high mammalians toxicity and also for the failure of insect control program. One of the main reasons for the failure of insect control program is that the insects develop resistance for prolongs use of same insecticides (Pieterse *et al.*, 1972). On the other hand, plant derived pesticides are more readily biodegraded. Therefore, they are less likely to contaminate the environment and less toxic to mammals (Freedman *et al.*, 1979). In view of these facts researchers have diverged their attention towards age-old practice of using phytochemicals, which would be of not hazardous, easy to use and specific in their action (Koul, 1982). Several insecticidal agents have already been isolated, identified and screened from members of plant families (Saxena, 1983). The toxic effect of caffeine and castor oil to *T. casteneum* adult and larvae has been reported by Mondal and Akhtar (1992).

The plant *Psoralea corylifolia* L., locally known as Buchkidana in Bangladesh, is a shrub, widely distributed in tropical countries and belongs to the family Papiolionaceae. The plant has considerable reputation for its medicinal value in traditional medicine. The rural people use the seeds are as laxative, stomachic, deobstruent, aphrodisiac, anthelmintic, diuretic, diaphoretic and it is also found to be effective against leucoderma, leprosy, psoriasis and other cutaneous affection (Drury, 1873). The seeds are also used internally to heal ulcer, asthma, heart troubles, scabies, biliousness, to cure blood diseases and elephantiasis (Anonymous, 1969). Previous biological screening of the compounds isolated from the plant *Psoralea corylifolia* showed remarkable antimicrobial (Kawl *et al.*, 1976), antitumor and cytotoxic (Byung *et al.*, 1998) and many other effects. With the aim of obtaining bioactive principles we have screened preliminarily for the antibacterial, cytotoxic effects of the crude chloroform extract of the seeds of *Psoralea corylifolia* and bioassay guided fractionation of this fraction led to the isolation of the compound 6-(-3-methylbut-2-enyl)-6′-7-dihydroxycoumestan (1). There is no report for the biological activity evaluation of the compound 6-(-3-methylbut-2-enyl)-6′-7-dihydroxycoumestan (1). This led to the present experiment, from the pest management point of view.

Materials and Methods

Isolation of compound (1): The matured seeds of the plant *Psoralea coryfolia* L. were purchased in Bangladeshi market during the month of March-April, 1998. Dried seeds were pulverized into powder (500 g) and extracted in a soxhlet apparatus with rectified spirit (3 L) at its boiling points. After concentration, the rectified sprit extract was diluted with water and defated with petroleum ether and then successively with chloroform and ethyl acetate (Khatune, 2000). The chloroform soluble fraction after drying and evaporation of the solvent was subjected to a column (Beckett and Stenlake, 1986) of silica gel of (60-120-mesh) eluted with n-hexane, chloroform and methanol by gradually increasing their polarity. Fractions 9 to 15 eluted with n-hexane and chloroform, showed identical spots on thin layer chromatography (TLC) and were combined. The combined fraction was subjected to preparative thin layer chromatography (PTLC) (Egon and Stahl, 1969) eluted with n-hexane: ethyl acetate (7:1). The major band glow under UV was scrapt of and after work up afforded needle like crystal (mp. 125-126°C) and was identified as 6-(-3-methylbut-2-enyl)-6′-7-dihydroxycoumestan (1) (Fig. 1) on the basis of its ^{1}H-NMR, ^{1}H–^{13}C correlation, ^{13}C–DEPT 135°, HMBC, HSQC spectra. This is a novel compound and used for the evaluation of its toxicity to *Tribolium casteneum* Herbst. adults and larvae.

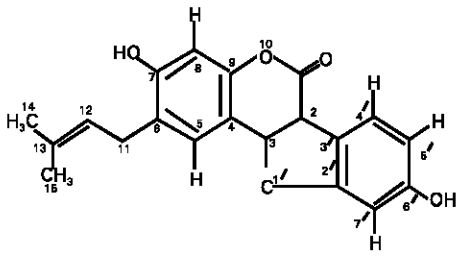

Fig. 1: 6-(-3-methylbut-2-enyl)-6′-7-dihydroxycoumestan (1)

Test insects: *Tribolium casteneum* beetles at different instars and freshly formed male and female of 12 days old, used in bioassay were reared in laboratory at 30 ± 2°C. These were maintained in 4-lb kilner jar containing standard food medium. A standard mixture of previously sieved wheat flour and powdered brewer's yeast (19: 1) (Park and Frank, 1948) was used as a food medium throughout the experiment. The food medium was sterilized at 120°C for 6 h in an oven and was not used for at least 15 days to allow its moisture content to equilibrate with the environment (Khan, 1981).

Larvicidal/insecticidal effects: The compound (1) was tested for its pesticidal action at different concentration, viz. 0 (control), 400, 800, 1200 and 1600 ppm after diluting the stock solution with dimethylsulfoxide (DMSO). The prepared doses were used in 5 replication each having 10 early 1st, 2nd, 3rd, 4th, 5th and 6th instars larvae and female and male adults of the insects in test tube with sample treated food media (0.5 mg).

The mortality of larvae at different instars as well as individual adults was assessed after 24, 48 and 72 h of treatment. Control group insects, reared in food media were treated with solvent only. The mortality was determined by counting survivors at the end of exposure period and the control mortality was adjusted by using Abbott's (1925) formula and results were subjected to probit analysis following the method of Busvine (1971). The mortality relationship was expressed as a median lethal dose (LD_{50}).

Results and Discussion

The results demonstrated that the compound (1) proved to toxic and a large number of larvae were killed. The compound caused the highest mortality of the 1st instar larvae in comparison with other larval instars (Table 1), which indicated that the newly hatched larvae were the most susceptible to the compound. The 1st instar larvae (after 72 h exposure) were the most susceptible with lowest LD_{50} value (388.45 ppm) whereas the male adult (after 24 h exposure) were less susceptible with highest LD_{50} values (2350.41 ppm). Thus the LD_{50} values of the samples were age dependent. This may clearly support of others that insects age play an important role in influencing susceptibility (Muwangi and Mukiama, 1988).

The graphs (Fig. 1a-h) showed that the mortality of larvae as well as individual adults increased as the doses of the sample were increased. Both larval growth and adult populations of *T. casteneun* were significantly reduced in (1) treated media in comparison with those of control. Although the mechanism of action is not known, but according to Miller *et al.* (1981) and Fox (1990) it may acts as chitin synthesis inhibitor. Due to its effect the integument of larvae much less extensible which might have the effect of increasing internal pressure in the body, restricting movement and hampering feeding. This change to the cuticle elasticity may also be responsible for decreased larval growth (Schneiderman and Gilbert, 1964).

The reduced adult population of both sexes of *T. casteneun* in the present experiment might also be due to the effect of the sample a chitin synthesis inhibitor. It inhibits the moulting and pupareum formation as a result, emergence of the adults is suppressed or incomplete and the ultimate result is the reduction of adult population (Miller *et al.*, 1981; Fox, 1990). The reduced population of the adult might also due to the larvicidal and ovicidal activity of the sample. The mechanism of action has been previously reported by some researchers against housefly, *Muska domestica* L. (Giga, 1987; Webb and Chapman, 1993).

Few works have been conducted on the effect of plant extracts and isolated compounds on *Tribolium* species (Jahan *et al.*, 1989; Khalequzzaman and Islam, 1992) and the present result is more or less similar to Khalequzzaman *et al.* (1998) and Jahan *et al.* (1989) who reported the insecticidal properties of tobacco leaf and bishkatali against *T. confusum* larvae and also similar to Mondol *et al.* (1992) who reported the insecticidal properties of castor oil against *T. casteneun* larvae and adults.

In our previous study we reported the synergic effects of plant

Table 1: Mortality data of *T. casteneun* larvae at different instars and adults treated with compound (1)

Life	Duration of treatment (h)	LD_{50} value (ppm)	95% confidence limits (ppm)		χ^2 value
			Lower	Upper	
Larvae					
1st	24	910.34	717.49	1155.04	1.73
	48	620.47	455.67	844.88	1.68
	72	388.45	215.72	699.47	0.16
2nd	24	1159.87	869.41	1547.35	2.99
	48	714.88	510.97	1000.16	2.78
	72	404.26	260.34	627.74	2.07
3rd	24	1395.70	970.57	2007.04	1.70
	48	740.75	540.13	1015.89	1.90
	72	493.97	312.61	780.53	0.24
4th	24	1605.34	1077.66	2391.40	1.00
	48	835.61	620.24	1125.74	2.57
	72	565.83	367.98	870.05	1.05
5th	24	1652.84	1029.86	2652.67	0.53
	48	916.79	695.55	1207.72	2.71
	72	729.50	536.04	992.79	1.10
6th	24	1764.32	1098.81	2832.91	0.94
	48	994.16	744.15	1328.15	1.88
	72	784.09	587.95	1045.66	0.86
Male	24	1678.52	1092.00	2580.06	1.30
	48	992.04	736.06	1337.03	3.92
	72	795.67	589.37	1074.18	1.80
Female	24	2350.41	1216.34	4541.83	1.24
	48	1395.70	970.57	2007.04	1.70
	72	985.12	751.07	1292.11	1.24

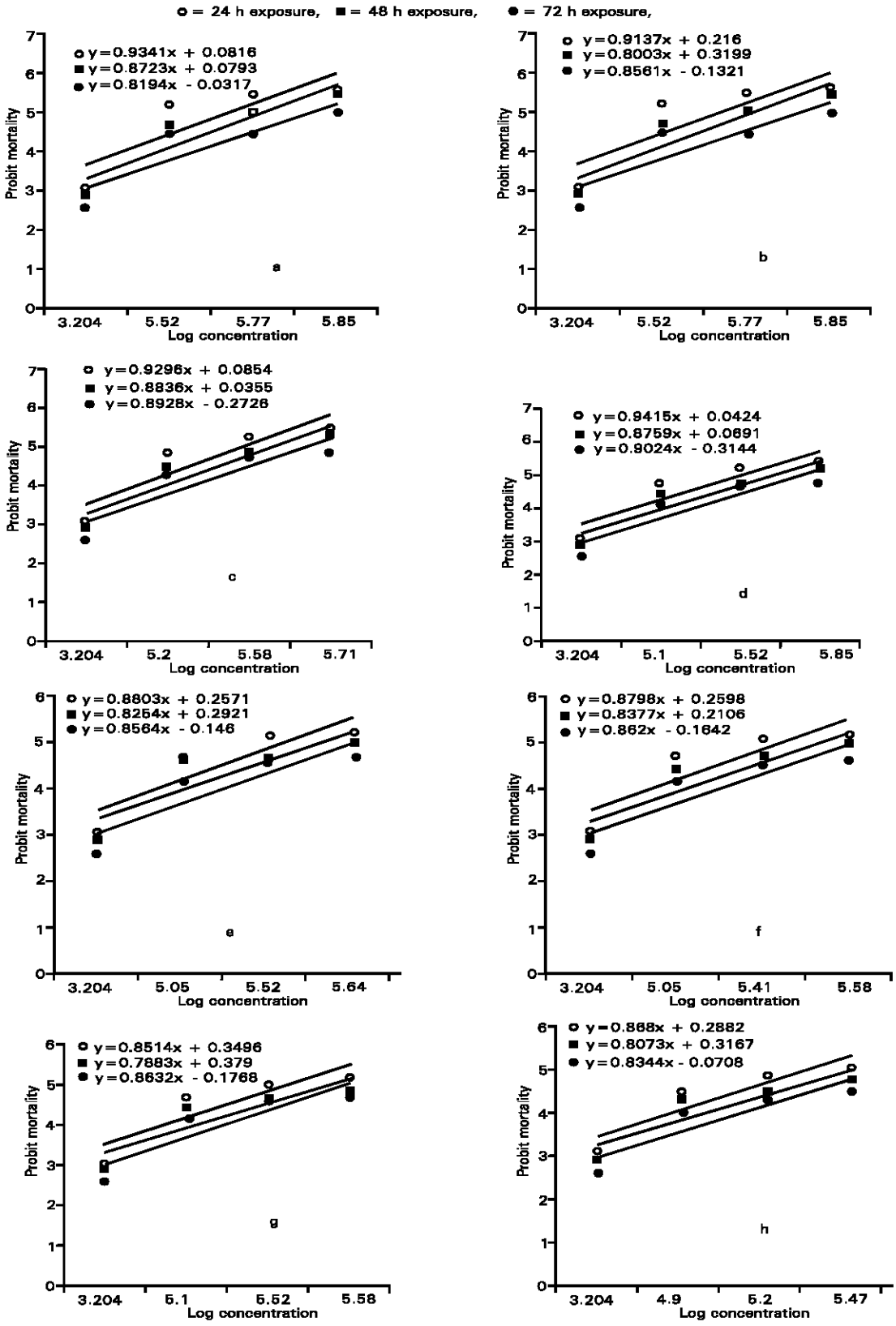

Fig. 1a-h: Regression line of probit mortality of *T. casteneum* on log dose of compound 1, a) 1st instar, b) 2nd instar, c) 3rd instar, d) 4th instar, e) 5th instar, f) 6th instar, g) male adults, h) female adults

extracts on common housefly *Musca domestica* (Baki *et al.*, 2002). In continuation of this study we selected the common botanical insect *Tribolium* and the results of these experiments indicated that the isolated compound 6-(-3-methylbut-2-enyl)-6′-7-dihydroxycoumestan may be used as effective botanical insecticides against *Tribolium*. Infestation of stored products by insects presents a serious problem. A large number of synthetic insecticides have been used against these pests. Unfortunately, these insecticides impart health hazards. Therefore, the use of hazard free insecticides is the foremost prerequisite for protection of stored products (Khanam *et al.*, 1991). Botanicals possess low mammalian toxicity. Thus the compound (1) with insecticidal property may serve this purpose, which is important from integrated pest management point of view. However, further investigation is to be directed towards this line with varying concentration, extracts of different parts of this plant on various *Tribolium* species.

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