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TOXICITY RESPONSE OF SYNTHETIC PYRETHROID AND A PLANT PRODUCT ON RED FLOUR BEETLE *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA : TENEBRIONIDAE)

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Abstract: *Tribolium castaneum* (Herbst) (Coleoptera : Tenebrionidae), commonly known as rust red flour beetle was an universal pest of stored produce with an extensive feeding diversity. The management of *T. castaneum* in field conditions was through fumigation and spraying of suitable insecticides.The response of *T. castaneum* adults and final instar grubs to a synthetic pyrethroid, deltamethrin and a plant product, leaf extracted of a tree, *Pongamia glabra* through topical application and oral administration The action of *P. glabra* was different from that deltamethrin in that, more concentration of the toxicant was required in oral administration than in topical application. The probit analysis of Finney (1971) was used to analyse the results of the toxicity studies. This regressional model was used to test the relationship between toxicant dosage and mortality after 24, 48, 72, 96 and 120h of exposure to the toxicants.

Keywords : *T. castaneum*, deltamethrin, *Pongamia glabra* , oral administration, topical application, probit analysis

Introduction

Management of *T.castaneum* was by using a synthetic pyrethroid, deltamethrin, as a topical spray on food bags (Yadav, 1986 and Sinha and Saxena, 2000). Toxicity of deltamethrin varied with the different life stages of *T.castaneum*. The grubs belonging to different age groups and the adult beetles reacted differently to the pesticides applied (Korunic and Koren, 1985; Arthur, 1997 and Karnataka, 2000). Similarly, the mode of application of the pesticide was important in determining the toxicity level. Topical applications (Sinha and Saxena, 2000) and consumption along with food as stomach poison (Yadav, 1986) produced observable variations in the mortality pattern.

As an alternative to the chemical pesticides, a number of botanicals were effectively used in the control of stored grain pests. The leaves of *Pongamia glabra*, a common perennial leafy tree, found in scrub jungles around the western ghats of southern India (77° 21'27"E and 8° 23'36"N) had been used as an insect deterrent and mild insecticide (Reddy *et al*., 1999; Pal *et al*., 2000 and Shoba and Thomas, 2001). This study was intended to compare the toxicity of deltamethrin and leaf extract of *P.glabra*. Toxicity response was studied both in final instar and adult *T.castaneum* through topical application and also by supplying the toxicant along with food to act as stomach poison.

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Materials and methods

The response of both adult and final instar *T.castaneum* to deltamethrin and *P.glabra* leaf extract was studied using the toxicity bioassay method of Finney, 1971.

Deltamethrin

Commercial grade deltamethrin liquid (2.8 E.C w/w), procured from the local pesticide retailer was used. Separate toxicity tests evaluating topical application and oral administration as stomach poision were conducted for the final instar larvae and adult beetles.

Topical application

Deltamethrin was diluted using double distilled water and 11 different concentrations for the adult (in % v/v :1, 2, 4, 6, 8, 10, 12,14, 16,18 and 20 - the active ingredients being-in mg/ml 0.28, 0.56.1.12,1.68, 2.24, 2.8, 3.36, 3.92, 4.48, 5.04 and 5.6 respectively) and 12 concentrations for final instar grubs (in% v/v : 0.1, 0.5, 1.0 ,2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 - the active ingredients being-in mg/ml 0.028, 0.14, 0.28, 0.56, 0.84, 1.12, 1.4, 1.68, 1.96, 2.24, 2.52 and 2.8 respectively), were prepared. For ease of calculation, toxicant concentration were expressed as v/v percentage.

For each exposure, 20 insects were taken in a wide mouthed transparent plastic screw cap bottle. Prior to the exposure the insects were transferred to a large glass plate on which, filter paper sheets were spread for absorbing the extra liquid sprayed. Only one topical application was given and the insects were douched with the pesticide solution for about 30 minutes. The insects were placed under a wire netted hood soon after spraying, preventing them from straying out. Then the insects were wiped off the moisture and transferred to labelled bottles containing wheat flour. The insects were inspected every 6h and mortality was recorded.

P.glabra

P.glabra leaves, plucked from the local scrub jungle were shade dried and ground in a blender. Exactly 100g of the leaf powder were extracted with 150ml double distilled water in a Soxlet extraction apparatus for about 4h. The extracted solution was diluted to obtain concentrations of 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32% for adult and 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24% for final instar larval *T.castaneum*. The diluted extracts were sprayed following the afforesaid spraying procedure.

Spraying apparatus

A glass atomizer sprayer was used for spraying the toxicants. Once spraying with a particular concentration was completed the apparatus was thorougly cleaned with hot glass distilled water and dried before filling the sprayer for further application.

Toxicant consumption

Separate replicates, each containing twenty adult or final instar *T.castaneum* were tested for the toxicity of deltamethrin and *P.glabra* leaf extract when the toxicants were fed to the insect along with food as stomach poison. From the deltamethrin solutions prepared by dilution on v/v basis, one ml was mixed with 50g of fine wheat flour. The dough obtained was dried in a hot air oven at 60° C and subsequently powdered using a mortar and pestle. The powder contained wheat flour with the toxicant imbibed. The equipments used for grinding flour were thoroughly cleaned after every individual use.

Deltamethrin was fed to adult *T.castaneum* in 12 concentrations (0.8, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0,7.0, 8.0, 9.0, 10.0 and 11.0% - the active ingredients being-in mg/g of wheat flour - 4.48×10^{-3}, 5.6×10^{-3}, 1.12×10^{-2}, 1.68×10^{-2}, 2.24×10^{-2}, 2.8×10^{-2}, 3.36×10^{-2}, 3.9210^{-2}, 4.4810^{-2}, 5.04×10^{-2}, 5.6×10^{-2} and 6.16×10^{-2} respectively and to final instar grubs in 11 concentrations (0.01, 0.02, 0.03, 0.04, 0.05, 0.06,

0.07, 0.08, 0.09, 0.1 and 0.11%- the active ingredients being - in mg/g of wheat flour - 5.610^{-5}, 1.1210^{-4}, 1.68×10^{-4}, 2.24×10^{-4}, 2.8×10^{-4}, 3.36×10^{-4}, 3.92×10^{-4}, 4.48×10^{-4}, 5.04×10^{-4}, 5.6×10^{-4} and 6.16×10^{-4} respectively.

P.glabra leaf powder was mixed with wheat flour in w/w basis to obtain concentrations of 30, 32, 34, 36, 38, 40, 42, 44, 46 and 48% for adult and 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22% for final instar *T.castaneum*. The life stages of *T.castaneum* were released into each of these containers and subsequently monitored every 6h and mortality recorded.

Probit analysis

The probit analysis of Finney (1971) was used to analyse the results of the toxicity studies. This regressional model was used to test the relationship between toxicant dosage and mortality after 24, 48, 72, 96 and 120h of exposure to the toxicants. Log dosage and probability of mortality values were calculated and based on a regressional equation of y = a + bx 'n' h. LC/ D_{50} values were obtained using the regression equation. The lower and upper confidence limits of the LC/D_{50} values were also calculated.

Results

The results of the probit analysis of the toxicity response of final instar *T.castaneum* to topical application of deltamethrin were much lesser for the grubs compared to the adults. After 24h, exposure to 10 percent deltamethrin resulted in 100 percent mortality, while the LC50 value was 7.313. When the exposure time was extended, comparatively lesser concentration of the toxicant brought about very high mortality. After 96h, 5 percent of deltamethrin produced 80 percent mortality of the grubs while the corresponding LC50 value was 3.075.

The probit analysis of the response of adult and final instar *T.castaneum* to oral feeding with deltamethrin indicated dosage dependent mortality of the exposed insects. Adults were more resistant, compared to the grubs. Higher mortalities were observed even in lower concentrations compared to topical application. 100 percent mortality occurred, 72h after, being fed with 8 percent deltamethrin ,while the LD50 value was 0.055. The grubs recorded 100 percent mortality, 24h after being fed, with 0.11 percent deltamethrin corresponding LD50 value was 0.315. After 96h, a dosage of 0.08 percent deltamethrin brought about 100 percent mortality,while the LD50 value was 0.036 (table1).

The probit analysis of the toxicity response of adult *T.castaneum* to *P.glabra* leaf extract showed that *P.glabra* leaf extract was much less toxic compared to the synthetic pyrethroid. A concentration of 32 percent of *P.glabra* leaf extract was required for 100 percent mortality of exposed adult *T.castaneum* over a period of 24h , while the LC50 value was 22.188.After 72h this concentration was 28 percent ,corresponding the LC50 value was 18.976. The final instar grubs too, were stronger in response to *P.glabra*. After 96h of exposure, 100 percent mortality occured in 16 percent concentration ,while the LC50 value was 16.480.

Feeding adult *T.castaneum* with *P.glabra* leaf extract was much less effective in producing high mortality. After 24h of feeding, 100 percent mortality of the beetles occured in 48 percent concentration, while the LD50 value was 43.328.Even after 96h, a dosage of 42 percent produced only 90 percent mortality,corresponding the LD50 value was 38.797. The final instar grubs were more responsive when fed with the leaf extract. The grubs showed 100% mortality, after 96h, at a dosage of 16%, while the corresponding LD50 value was 10.380.

Discussion

T.castaneum responded both to topical application and consumption of deltamethrin. The pesticide bioassay conducted on both adult and final instar larvae indicated 'partial kill' of the exposed

insects over different periods of exposure. Probit analyses of the toxicity bioassay was useful in finding out 'n' h LC_{50} values and their confidence intervals. The 24 h LC_{50} of deltamethrin topical application was much higher compared to the 96h LC_{50}. This type of toxicity response was regular and observed in a variety of organisms. Korunic and Koren (1985) showed that deltamethrin when mixed with piperonyl butoxide was highly effective against *S . oryzae* and *R.dominica* at a low dosage of 0.5 ppm. which was potent enough to protect stored wheat for 168 days. The same authors showed that pirimiphos-methyl at 4ppm was effective against *T.confusum*, which was resistant to deltamethrin. Yadav (1986) assesed the toxicity of wetable powder of deltamethrin against *T.castaneum* and showed that, a treatment of 1 kg. wheat seed with 3ppm deltamethrin dust controlled these insects for a period of 6 months. Sinha and Saxena (2000) showed that topical application of deltamethrin over the ventral tergite of adult *T.castaneum* at the rate of 0.5 ml. with the help of a micro applicator was the most effective bioassay technique for the measurement of deltamethrin toxicity. When the same toxin was administered as stomach poison along with food, there was a drastic decrease in LD_{50} levels indicating an increase in the toxicity of the chemical pesticide. The 96h LD_{50} in oral administration was 3.797 percent while the corresponding LC_{50} for topical application was 6.617 percent. Synthetic pyrethroids were extremely poisonous as stomach poison than as contact toxin. The same observation held good for the final instar grubs subjected to toxicant bioassay. Patourel and Singh (1984) showed that the toxicity of silca-pyrethroid admixture with wheat grain was highly toxic to *T.castaneum* since the pesticide was fed upon along with flour. The 48h LC_{50} of deltamethrin was 40± 2/µg/g of wheat. The 48h LC_{50} of deltamethrin (oral administration) recorded in this study was 5.52% (0.0309 mg/g of wheat flour) for adult *T.castaneum* and 0.075% (4.2×10^{-4} mg/g of wheat flour) for final instar. Similar studies by Grover and Mustafa (1992) showed that the LC_{50} of 7 analogues of monocrotophos to *T.castaneum* varied from 0.257 to 34.858 µg/cm^2. Arthur (1997) exposed adult *T.confusum* to cyfluthrin treated concrete and observed a post-exposure knock down of 82 percent.

In warehouses deltamethrin was sprayed on bags containing stored produce. The toxicant thus sprayed lingered on to the jute fabric acting as a deterrent, contact and stomach toxin. Soon after spraying, there was mass kill of beetles, straying out side the bags and mortality was observed in those staying inside also. The beetles or grubs in areas closer to the bag surface were the ones most affected. The beetles and the grubs usually preferred to stay in close vicinity to the outer surface of the bag. Yadav (1986) showed that a prophylactic spray of deltamethrin applied at 20 mg/m^2 on filter paper, jute fabric, cement, polyethylene film or mud surfaces was effective against *T.castaneum* and other stored produce pests for a maximum period of 170 days, the persistence of deltamethrin being greatest on jute surface leading to 100 percent mortality.

P.glabra leaf extract also acted as a contact and consumption toxicant. Being a plant product it was less toxic, compared to deltamethrin. The 96 h LC_{50} of *P.glabra* for topical application on adult beetles was 17.482 percent, about three times the quantity of the chemical pesticides needed to bring about an equivalent toxicity. Reddy *et al.*, (1999) used *P.glabra* seeds to protect green gram from pests during storage. A significant reduction in oviposition and adult emergence resulted in the pulse beetle C*allosobruchus chinensis*. Ahmad *et al.*, (2001) found that cholinesterase activity was inhibited in neem extract treated *T.castaneum* compared to an increase in cypermethrin treated beetles. Thus plant products seemed to be as potent as chemical pesticides. Williams and Mansingh (1993) reported the potency of *A. indica* against *T.confusum* by comparing the leaf extracts of sixty plant

Table 1 'n'h LC/D50 values to *T.castaneum*

S.No	Toxicant	Lifestage	Type of application	'n'h LC/D50 values (%)				
				24	48	72	96	120
1	Delta methrin	Adult	Topical	15.118	11.823	9.531	6.617	4.131
			Stomach	6.622	5.52	4.465	3.797	2.831
		Grub (final instar)	Topical	7.313	5.949	4.671	3.075	1.938
			Stomach	0.082	0.075	0.055	0.047	0.036
2	*P.glabra*	Adult	Topical	22.188	20.374	18.976	17.482	16.48
			Stomach	43.328	41.277	39.708	38.797	37.054
		Grub (final instar)	Topical	17.65	15.461	13.186	10.561	8.29
			Stomach	16.751	14.242	12.967	10.389	8.29

species by spraying a 10 percent (w/v) concentrate under a Potter's tower. Plant products were not inferior to chemical insecticides in controlling stored grain pests and they did not produce any damage to the ecosystem, the quality of grains or to human consumers of the treated stored produce. For the final instar grubs, the 96h LC_{50} was 10.561 percent, approximately 3 times higher than the equivalent deltamethrin toxicity. Plant products were comparatively less toxic and the common bioactive components were alkaloids and tannins. Martinez *et al.*, (2001) analysed the alkaloid and tannin contents of *Lupinus campestris* seeds and showed that alkaline thermal treatment reduced the concentration of these potent compounds with insecticidal action. These compounds also had antibacterial activities (Hou *et al.*, 2000). Any plant with sufficient alkaloid and tannin content could serve as a powerful inhibitor of insect pest, and these products were not very injurious to human beings.

When *P.glabra* leaf extract was orally administered to *T.castaneum* there was an increase in the amount of plant materials needed to bring about toxicity compared to topical application. This was contrary to the findings of the toxicity studies using deltamethrin. While a lower amount of deltamethrin was sufficient to kill as stomach poison, more amount of *P.glabra* leaf extract was needed to kill beetles, compared to the concentration of topical application for adult beetles. The 96h LC_{50} was 17.482 % (topical) while the 96 h LD_{50} was 38.797 percent (stomach). Such a trend was not observed in final instar grubs with closely placed LC_{50} and LD_{50} values. The 120h LC and LD_{50} values were the same (8.29 percent).

The adult beetles seemed to be hardier compared to the grubs. Pemonge *et al.*, (1997) found that topical application of *Trigonella foenum-graecum* L. at 6 and 30 µg/insect produced comparatively high mortality of about 23.3 percent in 10 days, while a 10 percent w/w ground material produced only 6 percent mortality. Thus, this plant product, like *P.glabra* was responsible for reduced mortality in oral administration and increased mortality in topical application and they could consume more of the leaf extract without any visible expression of toxicity response.

Use of plant products in the control of insect pests had been gradually increasing because of the less potent active ingredients, lower residual effect and lower incidence of resistance development. Pemonge *et al.*, (1997) reported that the use of plant allelochemicals was a promising alternative method for integrated pest management. When extracts acted directly on adults by contact, the powdered plant material inhibited reproduction, decreasing fecundity and exerting a larvicidal effect in *T.castaneum*. *P.glabra* could be a good substitute for the

common synthetic pyrethroid, deltamethrin. The leaves of *P.glabra* could be used for fumigation with smoke generated by burning the leaves or as stomach or contact poison for killing the beetles and their life stages. Dried leaves of *P.glabra* spread over storage bags could be a potent deterrent of *T.castaneum*. Rehuwanshi *et al.* (2001) reported the phagodeterrent activity of 6-dimethoxy ageratochromene isolated from weed *Ageratorum conyzoides* Linn. on *T.castaneum* adults, after 5 days of exposure to 3 percent concentration of the plant products. Similar phagodeterrent activity was observed when *T.castaneum* was supplied with food treated with the rhizome extract of the sweet flag, *Acorus calamus* (Chandel *et al.* (2001).

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