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Insecticidal Activity of Carbaryl and its Mixture with Piperonylbutoxide Against the Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) and their Effects on Acetylcholinesterase Activity

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Abstract: The insecticidal activity of carbaryl alone and its mixture with Piperonylbutoxide (PBO) was tested against males, females and larvae of the Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier), by both the feeding and the topical application methods. In addition, the Acetylcholinesterase (AChE) activity, the *in vitro* effect of carbaryl alone and the *in vivo* effects of carbaryl, PBO and their mixture on the AChE activity were estimated in RPW. A modification was carried out to enable estimation of AChE activity in this destructive pest. Tested materials were more toxic by feeding than by topical application and larvae were more susceptible than adults. In case of food treatment, the LC_{50} values of carbaryl alone were 1.183, 1.43 and 0.0367% for males, females and larvae, respectively. PBO had great synergistic effect on carbaryl toxicity, the LC_{50} values for carbaryl+PBO were 0.0155, 0.0253 and 0.00556% against males, females and larvae, respectively. The topical LD_{50} values for carbaryl and carbaryl+PBO against larvae were 27.1 and 14.4 μg/larva, respectively. The inhibition percentages of AChE activity in larvae fed on carbaryl and (carbaryl+PBO)-treated sugarcane were 0.8 and 64.4%, respectively. The results indicated the great role of oxidases in the reduction of carbaryl toxicity and their inhibition by PBO.

Key words: Red Palm Weevil, *Rhynchophorus ferrugineus*, acetylcholinesterase,carbaryl, topical application

INTRODUCTION

The Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), is the most dangerous and destructive pest of many palm trees in vast areas of the world[1-3]. Left with out rapid treatment, infested palm trees would die within 6-8 months[4]. Many control methods are used to combat Red Palm Weevil; however the main control method is the use of pesticides. Although organophosphate and carbamate insecticides have been used to control RPW for long time[5-12], there was not any attempt to estimate the effects of these pesticides on the acetylcholinesterase activity in this pest. Such biochemical studies are very useful to predict and discover the onset and appearance of resistance, to study the mechanism of resistance and to make use of synergists to overcome this problem or to potentiate the effect of certain pesticides against the target pest. The toxicity of these insecticides against the RPW by the topical application method has not been studied before. Therefor, this study was carried out to determine the toxicity of tested materials to RPW by both the topical application and the feeding methods and report on their effect on the AChE activity.

MATERIALS AND METHODS

This study was performed in the College of Agriculture, Department of Plant Protection, King Saud University, through 2003-2004.

Breeding the weevil colony: Young palm trees were longitudinally cut into two haves by the aid of an electrical saw. A deep groove (ca 10 cm width x60 cm long x15 cm depth) was dug in the middle of one half of the tree, 8 pairs of adult males and females were placed into the groove, then the tree two haves (containing the insects) were fixed together again using metal strips to secure the insects inside. Trees were kept inside big wire net boxes (70x70x150 cm) in the breeding room, at 27±3°C and 55-75% RH. Trees were opened after 23 days to get larvae, which were kept in plastic boxes with pieces of sugarcan to feed on, larvae with average weights of 3.5-4 g were used in bioassays. Prepupae and pupae were get after 50 days and were kept, individually, in 250 mL plastic jars; emerged adults were provided with split internodes of sugarcane to feed on; breeding of RPW on pieces cut from the stems of palm trees was proved to be

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successful[13]. Adults of 10-15 days old and 1-1.2 g average weights were used in bioassays.

Chemicals: Sevin, carbaryl 85% wp, was a product of Rhone-Poulenc, France; piperonylbutoxide, PBO (95%) was a gift from Saudi Chemical, Insecticide and Disinfectant Co. Ltd. (SCIDCO), Riyadh; acetylthiocholine iodide, ATChI (98.5% purity) was from BDH, England; 5, 5′-dithio-bis(2-nitrobenzoic acid), DTNB (98% purity) was from BDH; bovine serum albumin (>96% purity) was from Fluka AG, Switzerland and mercuric sulphate (99% purity) was a product of Merck.

Analysis of mercury: Mercuric sulfat (100 mg) was shaked with 10 mL distilled water, the concentration of Hg in the soluble fraction was found to be 47 ppm. The optical emission spectrophotometer, Optima 4300 DV-Perkin Elmer, was used for Hg analysis at 253.652 nm.

Bioassays

Feeding toxicity: Test concentrations were prepared by serial dilution of stock solutions in water or in aqueous PBO (0.5%), internodes of sugarcane with 12 cm length were immersed in the test concentrations for 1 min. After decantation off the pesticide solution over a wire mesh sieve, treated sugarcane was air-dried over filter papers for 1 h, longitudinally split internodes were used in case of adults; five pieces of treated food were placed into a 12x18x6 cm plastic box, along with five larvae or five adult males or females per box; the cover of the box contained 12 openings with 5 mm diameter; test concentrations were based on the active ingredient. Treated food was replaced with fresh, untreated one every 3 days. Each treatment was replicated 3 times, control food was immersed in PBO in water (0.5%). Boxes were kept at 25±1°C and 55-75% RH and dead insects were identified by losing any response to probing and were counted up to 15 days posttreatment. Probit analysis of data was carried out according to Finney[14].

Topical application treatment: Larvae and adults were immobilized by keeping them at 4°C for 5 min. All topical doses of carbaryl with or with out PBO (0.5%) were administered with the aid of a hand-operated microapplicator. An acetone solution of the appropriate insecticide preparation (5 μL/insect) was applied to the dorsum (behind the head) in case of larvae, or to the thoracic abdomen in case of adults; control insects were treated with PBO (0.5% in acetone). After the solvent evaporated, the treated insects were kept in plastic boxes (5 insects per box), with pieces of sugarcane to feed on. Three replicates of 5 larvae or adults each were performed for each dose; dead insects were counted up to 15 days, as described above.

Enzymatic studies: AChE activity in heads of larvae was estimated with the procedure of Ellman *et al.*[15] with a modification. Groups of 15 larvae were fed on sugarcane immersed in water, aqueous PBO (0.5%), 0.01% carbaryl in water and 0.01% carbaryl in aqueous PBO (0.5%), respectively. After 48 h, AChE activity was estimated in heads of controls and in heads of paralyzed larvae. Larvae were frozen in the deepfreezer, heads were separated, mouth parts were removed and the brown, hard, outer layer of heads were peeled off with the aid of sharp surgical blades. Heads were homogenized with cold phosphate buffer (0.1 M, pH 8, at 1 g per 8 mL). The homogenate was centrifuged at 1000 rpm for 5 min at 4°C; the supernatant was centrifuged at 15,000 rpm for 15 min using a Beckman J-12C centrifuge. The supernatant was decanted, kept on ice and used as the crude enzyme preparation. In 10 mL glass test tubes 200 μL of the crude enzyme was added to 2.56 mL of phosphate buffer (pH 8) and shaked for one min with 30 μL of the soluble fraction of mercuric sulfat (1.41 μg Hg); after that, 200 μL of DTNB (0.01 M in phosphate buffer (pH 7) was added; the reaction was initiated with the addition of 60 μL of ATChI (75 mM). Absorbance was recorded at 412 nm after 30 min using a Shimadzu UV-1201 spectrophotometer. Blank contained the same components except the substrate. ACHE activity was estimated, using the extinction coefficient 0f 13,600 M^{-1} cm^{-1} for the 2-nitro-5-thiobenzoate anion. Each treatment was replicated 3 times. Protein was determined by the method of Lowry *et al.*[16].

Why adding mercuric sulfat to the enzyme preparation before adding DTNB?

When we added DTNB to the enzyme preparation in the buffer medium, following the usual steps of the method of Ellman *et al.*[15] a rapid reaction leading to the formation of a deep yellow colour was noticed, which make this method invalid; trials to overcome this problem by diluting the enzyme preparation were unsuccessful; it was clear that the enzyme preparation was rich in component(s) with sulfhydryl groups, which react with DTNB, leading to the formation of the yellow anion, 2-nitro-5-thiobenzoate. Adding 5 μL (36.94 μg Hg) of aqueous mercuric chloride (1%) chelated the sulfhydryl groups but affected the enzyme activity; Adding 30 μL (1.41 μg Hg) of the mercuric sulfat solution was enough to chelate the sulphydryl groups and to keep the enzyme activity. It is concluded that the presence of the thiol groups in the enzyme preparation was the main reason

Table 1: Probit analysis for insecticidal activity of carbaryl and its mixture with Piperonylbutoxide (PBO) against Red Palm Weevil

		Feeding toxicity[a] (95% FL)			Topical application[b] (95% FL)		
Materials	Stage	LC_{50}	LC_{95}	Slope±SE	LD_{50}	LD_{95}	Slope±SE
Carbaryl	Larvae	0.0367 (0.0330-0.0409)	0.1173 (0.0948-0.1454)	3.26±0.08	27.1 (23.5-31.2)	127.3 (88.8-183.8)	2.45±0.056
	Males	1.1833 (0.9956-1.4062)	5.2652 (3.9719-6.9820)	2.54±0.10	>250	>250	-
	Females	1.4305 (1.2592-1.6249)	5.1978 (4.0806-6.6131)	2.94±0.10	>250	>250	-
(Carbaryl	Larvae	0.00556 (0.00494-0.00625)	0.0210 (0.0170-0.0270)	2.98±0.06	14.4 (12.8-16.1)	49.2 (39.7-61.1)	3.08±0.064
+PBO)	Males	0.0155 (0.0143-0.0167)	0.0339 (0.0294-0.0390)	4.83±0.16	>250	>250	-
	Females	0.0253 (0.0226-0.0283)	0.0723 (0.0536-0.0977)	3.61±0.16	>250	>250	-

[a] LC_{50} = carbaryl concentration (%) in which sugarcane was immersed, [b] LD_{50} = µg carbaryl per insect

for acetylcholinesterase activity not to be estimated in Red Palm Weevil before carrying out this study.

RESULTS AND DISCUSSION

The results in Table 1 indicate that larvae were more susceptible to carbaryl than adults, the LC_{50} values for larvae, males and females were 0.0367, 1.1833 and 1.4305%, respectively, which means that larvae is 39 fold more sensitive to carbaryl than females. Sensitivity of adult males and females were almost equal. This may be attributed to that larvae consume much more poisoned food than adults and/or to other factors.

The mixture of carbaryl+PBO was much more toxic to test insects than carbaryl alone, the LC_{50} values of the mixture against larvae, males and females were 0.00556, 0.0155 and 0.0253%, respectively, that is, PBO had synergised the effect of carbaryl against these stages by 6.6, 76.3 and 56.5 fold, respectively. Carbaryl was found to be effective against the different stages of RBW at the concentration of 1%[5,6,11].

Attempts to estimate the LC_{50} values for adult males and females by topical application with carbaryl alone or combined with PBO were not completely successful; adult mortality was less than 50% at the highest tested dose, 250 µg/insect; this means that each insect should come in contact with 5 µL of a spray solution of more than 5% carbaryl to achieve 50% kill. Larvae were more sensitive to carbaryl by topical application than adults, the LC_{50} for larvae was 27.1 µg/insect. Coadministration of PBO with carbaryl increased the toxicity of the insecticide by 2 fold, the LC_{50} of the mixture was 14.4 µg/insect (Table 1). The low topical toxicity of carbaryl, alone or combined with PBO, in case of adults and the higher toxic effect in case of larvae, may be attributed to a poor cuticular penetration in adults and a higher cuticular penetration in larvae. This assumption needs further studies for rates of cuticular penetration after topical treatment with radiolabeled carbaryl.

Table 2 indicates the *in vivo* effects of tested materials on the AChE activity in heads of Red Palm Weevil larvae. Carbaryl alone had no effect on the enzyme activity at the test concentration (0.8% inhibition only, Table 2).

Table 2: Activity of AChE in RBW larvae fed on sugarcane immersed in water, 0.01% carbaryl, 0.5% PBO and 0.01% carbaryl+PBO (0.5%)

Treatments	Control	Carbaryl	PBO	Carbaryl+PBO
Specific activity[a]	27.66	27.44	59.74	9.86
% inhibition	0.00	0.80	-115.98[b]	64.40

[a]specific activity = µmole ASCH per mg protein per 30 min
[b]PBO = Activated AchE

PBO alone had activated the enzyme (Table 2), this result is in good agreement with Solomon *et al.*[17] who reported that PBO had activated AChE in the Topmouth Gudgeon (*Pseudorasobora parva*) and attributed the increase in the acetylcholinesterase activity to induction of the enzyme by PBO. When PBO was coadministered with carbaryl, the insecticide inhibited the enzyme activity by 6o. 4% (Table 2), we recorded 4 and 68.7% mortality in larvae fed on sugarcane treated with 0.01% carbaryl and 0.01% carbaryl+0.5% PBO, respectively (unpresented data). *In vitro* trials resulted in I_{50} and I_{95} values of $1x10^{-5}$ and $31x10^{-3}$ M carbaryl, respectively, It is clear that PBO had blocked the detoxification of carbaryl by microsomal oxidases. The nature of the thiol groups found in the enzyme extract should be studied, it may be a key to important approaches for RPW control in the future.

After measuring the AChE activity in RPW, the way is paved now for more study with the rest of pesticides used to control the weevil and to make use of PBO and other synergists in this field.

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