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RESEARCH ARTICLE

ACETYLCHOLINESTERASE ENZYME LEVEL AND INSECTICIDE RESISTANCE STATUS OF ADULT BANANA PSEUDOSTEM WEEVIL, *ODOIPORUS LONGICOLLIS* OLIV. (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

The present study is to evaluate the effect of carbosulfan on the adult banana weevil *Odoiporus longicollis* Oliv. and also to assess the status of the acetylcholinesterase after treatment with sublethal doses of carbosulfan. Treatment with different concentrations of carbosulfan provided an LD₅₀ of 0.876 µg/µl and LD₉₀ of 1.117 µg/µl at 24 hr duration of exposure. The levels of acetyl choline esterase assays were conducted in tissues like gut, reproductive organ, fat body and the whole insect and quantified at various sublethal concentrations against different time duration of exposure. The adult insects were treated with 10%, 50% and 80% of lethal dose (LC₅₀) for 24 hr, 48 hr. and 72 hr. The results obtained in the present investigation clearly indicate that various concentrations of the carbosulfan have produced intensive toxic effect at tissue level in *Odoiporus longicollis*.

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INTRODUCTION

Banana pseudostem weevil, *Odoiporus longicollis* Oliv. is a pest of banana, plantain (*Musa* sp.), and ensete (*Ensete* sp.) and one of the major constraints for banana production in most of the countries. The banana weevil infestation decrease root growth, nutrient uptake and plant vigour leading to small fruit bunches and yields and weaken the overall stability of the plant. Infestations in newly planted fields results in crop failure. As life cycle of the pest may be completed within the pseudostem, it is very difficult to manage the pest. Banana pest management is an important activity in commercial banana production programme. Chemical control measures are the method for managing the pest. The stem injection of a systemic organophosphorus compound, monocrotophos is extensively used in controlling the pest (Sathiamoorthy *et al.*, 1998). As well as stem injection, other insecticide application methods may also be used, such as swabbing along with surfactants, swabbing with mud slurry containing the insecticide (Mathew *et al.*, 1997).

The region or site of egg laying on pseudostem needs to be covered by insecticidal spray to prevent egg laying (Dutt and Maiti, 1971b). Dutt and Maiti (1972b) observed maximum mortality of adults when exposed to contact insecticides or when adults come in contact with insecticide treated soil. Carbaryl 50 WP at 0.1 per cent spray also gave good results (Isahaque, 1978). Application of phorate or carbofuran @ 25 g/plant at the basal region upto six months from planting is effective in controlling the pest (Visalakshi *et al.*, 1989) which is the current practice of chemical control and is regarded by farmers as easy to manage, fast acting and effective. Use of chemicals such as dieldrin, endosulphan and fenitrothion against banana weevil infestation in Tanzania has met little success (Bujulu *et al.*, 1983). However, chemical control is reported to provide a short-time solution to the banana weevil problems while its long-time application resulted in weevil resistance (Gokool *et al.*, 2010; Bortoluzzi *et al.*, 2013; Bwogiet *et al.*, 2014; Aby *et al.*, 2015a). Chemical insecticides cause unwanted long-term effect, including insecticide resistance (Gold and Messiaen, 2000), pest resurgence, pest outbreak, ground water contamination and radical effects on beneficial insects (David, 2008). However, very little work has been done on screening for banana weevil resistance. Available reports (reviewed by Pavis & Lemaire, 1997; Kiggundu *et al.*, 1999; Gold *et al.*, 2002) were inconclusive

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defining resistant clones. Insecticide resistance is one of the major obstacles to the successful cultivation. When naturally occurring, genetic variation allows a small proportion of the population to resist and survive the effects of the insecticide to develop resistance. Host plant resistance offers a safe and long-term control strategy for the banana weevil within the framework of integrated pest management (Seshu-Reddy & Lubega, 1993).

MATERIALS AND METHODS

Odoiporus longicollis Oliv. (Coleoptera: Curculionidae) Banana pseudostem weevil (BSW), *Odoiporus longicollis* Oliv. (Coleoptera: Curculionidae) is an important pest of banana, and plantain. The adult weevil is black and measures 23-39mm in size. Many adults live for 1 year, while some survive up to 4 years. On moist substrates, the weevil can survive without feeding for several months. The sex ratio is 1:1 and oviposition rates of more than 1 egg/day have been recorded. The pre-oviposition period is 15-30 days. Gravid females lay yellowish white, elliptical eggs by inserting the ovipositors through ovipositional slits cut by the rostrum on the outer epidermal layer of the leaf sheath of the pseudostem down to the air chambers. Oviposition takes place only in the leaf sheaths. The number of eggs deposited is considerably reduced as the number of weevils increases, indicating the existence of a spacing pheromone, epideictic compounds which act as a deterrent to conspecific females (Ranjith and Lalitha 2001).

Collection of the insects and culture maintenance: Insects for the study were collected from Annassery, Vellalassery, Feroke, Chathamangalam, Nadakkavu, Kuttiady and Thottilpalamareas in Kozhikode district, Kerala, India. From the damaged banana stems, adults and larvae were collected and maintained in the lab. Insects required for the experiment were drawn from the culture just before the experiment.

Estimation of LD₅₀ and LD₉₀. Wide range of concentrations of carbosulfan is applied topically on the adults of *Odoiporus longicollis*. Ten replicates were kept for experiment and control set. Mortality after 24 hr was observed and recorded. To determine LD₅₀ and LD₉₀ a log dosage probit mortality regression line using statistical analysis by Finney (1971) was used. Repeated the bioassays three times using different batches of insects.

Acetylcholinesterase assay: The assay was conducted to quantify the levels of acetyl choline esterase. The enzyme assays were conducted in tissues like gut, reproductive organ, fat body and also the whole insect. Insects were cut laterally and tergum was removed. Alimentary canal, fat body and reproductive organs of adults were taken out separately. They were transferred to eppendorf tubes kept on ice. Tissues from 10 insects were pooled every time to get sufficient quantity. The adult insects were treated with 10%, 50% and 80% of lethal dose for 24 hr, 48 hr. and 72 hr. Statistical analysis was performed on the data observed at each level using Statistical package SPSS 20.0.

RESULTS

Different concentrations in µg/µl and the percentage mortality of the adult insect were recorded for the bioassay.

The concentrations applied were 0.7µg/µl, 0.8 µg/µl, 0.85 µg/µl, 0.9 µg/µl, 0.95 µg/µl, 0.98 µg/µl, and 1.0 µg/µl and the percentage mortality observed were 25, 37.5, 39, 40, 50, 70, and 99 respectively. LD₅₀ for the experiment was 0.876µg/µl and LD₉₀ obtained was 1.117µg/µl. (Table 1)

Acetylcholinesterase assay: The toxicity of organophosphorus and carbamate esters to animals is attributed to their ability to inhibit acetylcholine esterase, which is a class of enzymes that catalyzes the hydrolysis of the neurotransmitting agent acetylcholine (Ach). Its inhibition causes death, so irreversible inhibitors have been developed as insecticides such as organophosphates and carbamates (Aldridge, 1952). Acetylcholinesterase activity on the whole body of the target insect tested after treatment with sublethal concentrations of carbosulfan at various time intervals are provided in figure 1. Acetylcholine esterase activity (µ mol/ min / mg protein) in adult insects on application of 10% (0.1µg/µl) of insecticide LD₅₀ are 2.57±0.002, 1.161±0.03 and 0.766±0.03; and that on application of 50% (0.4µg/µl) of insecticide LD₅₀ are 2.35±0.001, 0.921±0.002 and 0.901±0.003 and the acetyl choline esterase activity (µ mol/ min / mg protein) on application with 80% of LD₅₀ are 2.03±0.001, 0.866±0.003 and 0.859±0.002 for 24hr, 48hr, 72hr time interval respectively.

Figure 2 provides the activity of AchE on fat body of the target insect. Acetyl choline esterase activity (µ mol/ min / mg protein) in the fat body of the adult insects on application of 10% (0.1µg/µl) of insecticide LD₅₀ are 1.376±0.02, 1.482±0.02 and 2.76±0.12; and that on application of 50% (0.4µg/µl) of insecticide LD₅₀ are 0.853±0.006, 1.46±0.001 and 2.884±0.05 and the acetyl choline esterase activity (µ mol/ min / mg protein) on application with 80% of LD₅₀ are 0.596±0.001, 0.625±0.015 and 1.33±0.003 for 24hr, 48hr, 72hr time interval respectively. Activity of AchE on reproductive organ of adult insects is provided in figure 3. Ach E activities for 10% of lethal dose application on the reproductive organ of insect were 0.854±0.02, 1.223±0.01 and 1.288±0.08; and that of 50% lethal dose application are 0.723±0.21, 1.07±0.006 and 1.139±0.004 and AchE activity for 80% lethal dose application are 0.535±0.006, 0.932±0.001 and 0.884±0.003 for the different time duration of 24hrs, 48hrs, and 72hrs respectively. The data on the figure 4 shows the activity of Ach E on gut of the adult insects when treated with different sub lethal concentration of carbosulfan for different time intervals. Acetyl choline esterase activity (µ mol/ min / mg protein) in the gut of the adult insects on application of 10% (0.1µg/µl) of insecticide LD₅₀ are 1.97±0.07, 1.62±0.03 and 0.985±0.01; and that on application of 50% (0.4µg/µl) of insecticide LD₅₀ are 1.89±0.003, 1.152±0.001 and 0.916±0.001 and the acetyl choline esterase activity (µ mol/ min / mg protein) on application with 80% (0.8µg/µl) of LD₅₀ are 1.782±0.002, 1.142±0.003 and 0.906±0.004 for 24hr, 48hr, 72hr time intervals respectively.

DISCUSSION

Pesticides and toxicants always produce a systemic effect on both target and non-target organisms, which could be identified by studying the mobilization of different key substrates and by following the activity of important enzymes.

Table 1. 24 hr LD₅₀ (µg/µl) of carbosulfan treated against the adult *Odoiporus longicollis* and its associated statistics

24 hr Lethal Dose	Lower - Upper limit	Regression equation	Degree of freedom	Chi square	Significance
LD ₅₀	0.876	Y=10.643x+8.9286	5	56.786	p<0.05
LD ₉₀	1.117	Y=10.643x+8.9286	5	56.786	p<0.05

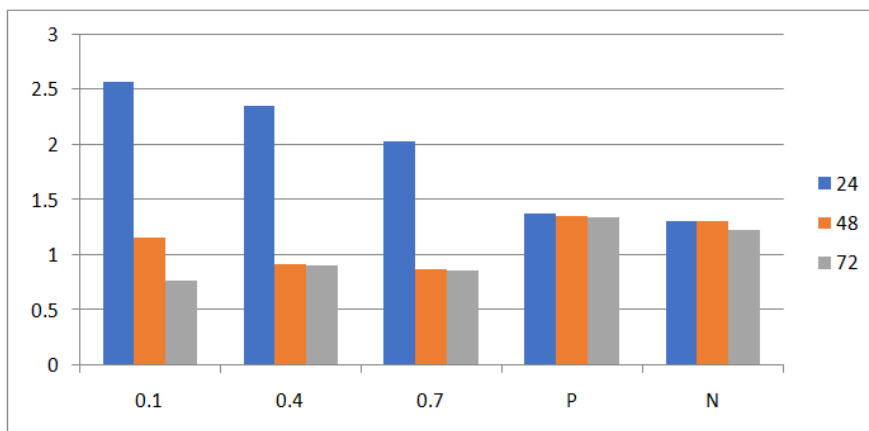


Figure 1. Acetyl choline esterase activity of the whole body of adult insect after treatment with different sub lethal concentrations of carbosulfan at different time duration

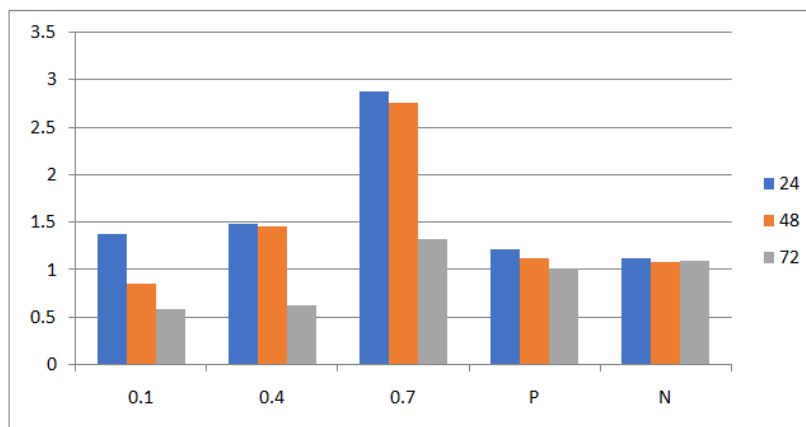


Figure 2. Activity of acetyl choline esterase activity of the fat body of adult insect after treatment with different sublethal concentrations of carbosulfan at different time duration.

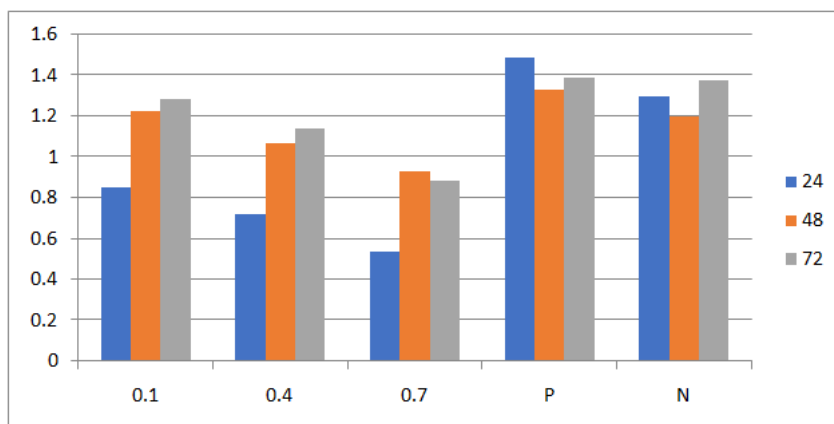


Figure 3: Acetyl choline esterase activity of the reproductive organ of adult insect after treatment with different sublethal concentrations of carbosulfan at different time duration.

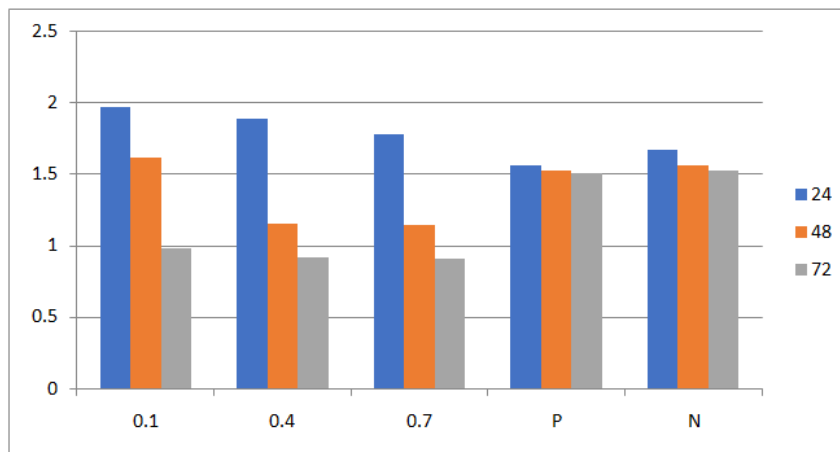


Figure 4: Acetyl choline esterase activity of the gut of adult insect after treatment with different sublethal concentrations of carbosulfan at different time duration.

Chemical control of insect pests is the most dominant approach at present. Toxicants in sub lethal dosages produced changes in the biochemical response of organisms leading to a change in physiological functions (Simpson and Raubenheimer, 1993). The results obtained in the present investigation clearly indicate that various concentrations of the carbosulfan have exerted intensive toxic effect at tissue level in *Odoiporus longicollis*. In insects, AChE has mainly been studied in relation to insecticide resistance because the enzyme is the target of organophosphate and carbamate insecticides and its insensitivity to insecticides is one of the major factors accounting for resistance; thus, these enzymes are used as reliable markers to assess the impact of toxic compounds on insects. Acetylcholinesterase (AChE) is the target of both organophosphate and carbamate insecticides. It is responsible for neurotransmitter degradation at the cholinergic nerve synapse. In the adult whole-body samples at 24hr exposure, the AChE activity is increased compared to control samples. But AChE activity is inhibited at 48hr and 72hr time interval. Compared to other tissues, fat body showed the highest AChE activity. This may suggest the involvement of metabolic resistance mechanism in this insect population. The AChE is inhibited in the sample of reproductive organs, compared to control samples. In insect gut samples, only the 24hr incubation samples showed an increased level whereas others got inhibited compared to control samples.

Conclusion

The target site for carbamate insecticides is acetylcholinesterase and these insecticides block the nerve transmission leading to the death of the insects. Increased level of the enzyme shows that it is less sensitive to the insecticide leading to resistance. A major type of antiChE insecticide resistance is selection for mutations conferring reduced organophosphorus and/or carbamates sensitivity, first noted in spider mites (Smitsaert, 1964) with well over 20 examples in insects involving at least 14 specific identified mutations (Oakshott *et al.*, 2010; Villatte *et al.*, 2000; Fournier *et al.*, 1993 and Fournier, 2005).

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