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Duration of Exposure and Mortality of Different Strains of *Prostephanus truncatus* (Horn) (Coleoptera: bostrichidae) Exposed to Bifenthrin Insecticide in the Laboratory

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Abstract

The length of exposure time to Bifenthrin on four strains of larger grain borer *Prostephanus truncatus* Horn (Coloeoptera: Bostrichidae) was studied in the laboratory at 28 -33^{0}C and 70 – 85% relative humidity. Five serial dilutions of the toxicant ranging from 0.031 to 0.0012 g/ml, including a control were used. Mortality was recorded at 3 hourly intervals of 3, 6, 9 and 12 hours post-treatment. Data were analysed using $\log_{10}$ versus probit regression and analysis of variance (ANOVA). Results showed that significant ($P<0.05$) dosage-related mortality responses were recorded at the different time intervals during the first 3 and 6 hours of exposure. Nevertheless, significant mortalities ($P<0.05$) occurred after 9 to 12 hours post-contact with the insecticide. The Ibadan strain appeared to be more susceptible with LD_{50} value of 10.19 µg/ml compared with the other strains, while the Benue strain showed more tolerance to the test insecticide with LD_{50} of 29.60 µg/ml. Thus, the Ibadan strain was two times more susceptible than the Enugu and Ghana strains and 3 times that of the Benue strain.

Keywords: *Prostephanus truncatus* strains; mortality responses to Bifenthrin.

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1. Introduction

The Larger Grain Borer (LGB) *Prostephanus truncatus* Horn was thought to be native of Central America. It was accidentally introduced from Central America into hot Tabora region of Tanzania in the late 1970s and was found attacking farm-stored maize and dried cassava causing severe losses [1]. It subsequently spread widely within Tanzania and into southern Kenya, Burundi and Malawi [2] and has spread to other countries in the region. In West Africa, a serious outbreak was first found in Togo in the early 1984 [3], Ghana in 1989 [4] and Nigeria in 1992 [5]. It has now spread to many African countries, becoming the most destructive pest of stored maize and dried cassava in both West and East Africa.

The use of synthetic insecticides dominated initial attempts to control *P. truncatus* for years. Products from virtually all classes of insecticides have been used, including organochlorines, organophosphates, carbamates and Pyrethroids [6]. The earliest report of insecticides testing against *P. truncatus* came from Central America and Mexico. Researchers in [7] investigated the protection of maize grain using Dichloro-diphenyl trichloroethane (DDT), Benzene hexachloride (BHC), magnesium oxide, and chlordane, and observed that all treatments gave good protection at 10 months of storage. Unfortunately, the use of DDT and related compounds has been banned. Good results have also been obtained using primiphos-methyl, fenithrothion and bromophos.

In Africa, the first field trails which tested the admixture of diluted dusts of pirimiphos-methyl, fenitrothion and bromophos to shelled maize showed that only pirimiphos-methyl maintained the grain in good condition [8]. However, Pyrethroids gave the best result. Magnesium phosphide fumigation also gave good results. Consequently, they recommended that maize should be shelled and admixed with diluted dust of permethrin before storage.

Pyrethrins are insecticides that are derived from the extract of *Chrysanthemum cinerariaefolium* (pyrethrum) flowers [9]. The plant extract, pyrethrum, contains Pyrethrins . Pyrethroids are synthetic forms of the naturally occurring pyrethrins obtained from pyrethrum, the oleo-resin extract of dried *Chrysanthemum* flower. The insecticidal properties of pyrethrins are derived from the ketoalchoholic esters of chrysanthemic and pyrethric acids. These acids are strongly lipophilic and rapidly penetrate many insects and paralyze their nervous system [9]. There are two types that differ in chemical structure. Type-1 Pyrethroids include allethrin, tetramethrin, resmethrin, d-phenothrin, bioresmethrin and permethrin. While Bifenthrin and deltamethrin are type-2 Pyrethroids [9]. Both types 1 and 2 Pyrethroids inhibit the nervous system of insects and produce paralysis. This occurs at the sodium ion channels in the nerve cell membrane. Some type-2 Pyrethroids also affect the action of the neuro-transmitter called GABA (γ-aminobutyric acid) [9].

Pyrethrins are extremely sensitive to light, heat and moisture. However, the Pyrethroids, the synthetic analogues were developed to capture the effective insecticidal activity of this group of botanical insecticides with increased stability in light, thus yielding longer residue time [10].

Recent developments concerning Pyrethroids are quite remarkable. They are fast becoming the most potent group of synthetic insecticides ever to enter the market. With the modification of both alcohol and acid components, they are also becoming stable enough to be used for agricultural purposes [11].

The synthetic pyrethriod compounds permethrin, deltamethrin, phenothrin and fenvalerate have been found to be more effective than organophosphorus compounds in causing adult mortality of *P. truncatus* and reducing the development of succeeding generations [8]. The recommended control measures, for protecting maize stored on farms in areas of Tanzania affected by *P. truncatus*, include shelling maize after harvest and applying permethrin dust at the rate of 50g of 0.5% dust per 90 kg grain (the content of a new standard sack) [12]. Although this treatment reduced storage losses caused by *P. truncatus*, it led to an increase in the importance of damage caused by *S. oryzae* (Linnaeus) and *S. zeamais* Motschulsky and other indigenous insect pests [13].

Bifenthrin is a synthetic pyrethriod that affects the nervous system of insects. Studies show that it is relatively insoluble in water. Its half life in soil ranges from 7 days to 8 months depending on the soil type. Bifenthrin is one of the few synthetic Pyrethroids that are relatively stable in direct sunlight, and valued for its broad spectrum control that includes plant sucking bugs, aphids , weevils and leaf-eating Lepidoptera among others [14].

Early laboratory experiment on Bifenthrin in Queensland, Australia was conducted with the aim of identifying effective application rates of Bifenthrin for control of two species: the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) and lesser grain borer, *Rhyzopertha dominica* (F.). *O. surinamensis* was chosen because resistance to the organophosphates (fenitrothion, primiphos-methly and chlorpyriphos-methyl) was becoming common. *R. domonica* was chosen because some population had appeared in Queensland that was resistant to the pyrethriod, bioresmethrin. An application rate of 0.5 mg/kg of Bifenthrin gave complete control of susceptible and resistant *O. surinamensis* in freshly-treated wheat. This rate also gave complete control of susceptible and Malathion-resistant *R. dominica* but not *R. dominica* that was resistant to both malathion and bioresmethrin. Even the addition of a synergist, piperonyl butoxide, did not improve bifenthrin efficacy against the bioresmethrin-resistant *R. dominica.* Recently, [15] reported that Bifenthrin was three times more toxic to adults of *P. truncatus* than *Aloe vera* leaf powder with 50% lethal dose of 6.06 mg/ml.

The present studies therefore, aimed at evaluating the mortality responses of different strains of *P. truncatus* and the effects of length of exposure time to residual applications of Bifenthrin.

2. Materials and Methods

2.1 Rearing of P. truncatus From Three Agricultural zones in Nigeria and a Strain from Ghana

Prostephanus truncatus adults were collected from three different locations in Nigeria namely, Ogbete-Enugu, Markurdi-Benue and Dugbe-Ibadan, representing, South-East, North-Central and South-Western Agricultural zones of Nigeria and a strain collected from Legon, Accra Ghana. These strains of the insect were reared on a standard maize variety, white Mangu Jos obtained from Ogbete market in Enugu. The maize was placed in an oven and heat sterilized at 70^0C for three hours to kill any existing infestation. When cooled, 500g of the maize

were measured into one litre kilner jars and sterilized at 60^0C for three hours. Fifty to one hundred adult insects were introduced into each jar. The whole set up was placed in a large basin containing vegetable oil to prevent the entry of other insects into the culture. The culture was allowed to stand under ambient laboratory conditions for two months to obtain enough F_1 progeny. These populations maintained in the laboratory were used for the final study.

2.2 The Insecticidal Treatments

The pyrethriod formulation, Bifenthrin (10% WP) (Bistar) was obtained from Dizengoff Chemical Company, Enugu. Five dosages namely 0.031 g/ml, 0.016 g/ml, 0.0058 g/ml, 0.0025 g/ml and 0.0012 g/ml were serially diluted in acetone (Analar grade) using standards by [12].

2.3 Residual Application of the Insecticide to Determine Lethal Doses.

No. 1 Whatmann filter paper measuring 9 cm in diameter was placed in each of the Petri-dishes used for the experiment. The various concentration levels of the insecticide used included 0.031 g/ml, 0.16 g/ml, 0.005 g/ml, 0.0025 g/ml and 0.0012 g/ml and each was replicated three times. Aliquots of 0.5ml of each concentration was evenly spread onto the filter paper and left for about an hour to ensure proper spreading of the solution and allow the acetone to evaporate completely. Control treatments with plane acetone were included. Subsequently, 10 unsexed one to seven-days old adults were introduced into each Petri-dish using a mouth-operated aspirator. Each of the Petri-dishes was covered with its lid to prevent escape of the insects. The layout plan and design of the experiment was a randomized block design and the whole set up was left on a laboratory bench. Mortality counts were taken for 12 hours at 3 hourly intervals. Both moribund and dead insects were counted to determine mortality rates.

2.4 Statistical Analysis

Correction of mortality data obtained was carried out using the formula in [16]: PT = (Po – Pc/100 –Pc) x 100; where PT is the percentage (%) corrected mortality, Po is the observed mortality and Pc is control mortality. Log_{10} versus probit regression analysis was carried out [17] in determining LD_{50}. The data were analyzed using factorial ANOVA model in SPSS version 17 for Windows Statistical Package [18].

3. Results

The mortality response of *P. truncatus* from the different locations exposed to residual application of Bifenthrin at 3 hourly intervals are presented in Table 1. It should be noted however, that the Ibadan strain of the insect appeared to be heavier in body size than the others. The results show that Bifenthrin exhibited significantly ($P<0.01$) different levels of toxicity to *P. truncatus* strains. The mortality responses were dose-dependent though the mean percentage mortality responses were respectively, 53.4±14.8%, 70.1±17.3%, 50.0±17.2% and 57.5±13.3% for the Enugu, Ibadan, Benue and Ghana strains. Mortality also increased with increasing concentrations of insecticide and also time-dependent, indicating that the responses increased with increasing

duration of exposure to the insecticides. In terms of their relative susceptibilities to Bifenthrin, the Benue strain was found to be about three times more tolerant than the Ibadan strain.

The mortality responses of the Enugu strain of *P. truncatus* (Table 1) showed there that were no significant (P>0.05) differences during the first 3 and 6 hours of exposure to Bifenthrin (Least Significant Difference (LSD) = 13.56). However, significant mortalities (P<0.05) occurred after 9 and 12 hours of contact with the insecticide.

Table 1: The mean percentage mortality effects of different concentrations of **Bifenthrin** on adults of *P. truncatus* from **Enugu** at 3 hourly intervals.

Conc. (µg/ml)	**3 hrs**	**6 hrs**	**9 hrs**	**12 hrs**	**Mean ±s.e**
31.3	26.7	43.4	50.1	94.4	53.4±14.8
15.6	23.3	26.6	46.6	73.3	42.5±11.7b
5.0	20.0	23.3	43.3	66.6	38.3±10.9b
2.5	10.0	13.3	29.9	36.6	22.5±7.5a
1.2	6.7	6.7	16.7	23.4	13.4±4.8a
Mean ± s.e	**17.3 ± 4.1a**	**22.7± 6.4a**	**37.3 ± 6.4b**	**58.9 ± 12.4c**	
Control	**0**	**5**	**0**	**0**	**1.3**

Means of three replicates (± s.e), LSD = 13.56, Means followed by the same letter are not significantly different.

The mortality responses of the Ibadan strain (Table 2) was significantly higher (LSD = 19.88; P<0.05) at the 6th and 9th hours. However, the LSD shows that the mean responses at the 12th hour were not actually different from those at the 9th hour. The Benue strain (Table 3) showed significantly higher responses (P<0.05; LSD = 14.46) at every additional three hours exposure to Bifenthrin.

Table 2: The mean percentage mortality effects of different concentrations of **Bifenthrin** on adults of *P. truncatus* from **Ibadan** at 3 hourly intervals.

Conc. (µg/ml)	**3 hrs**	**6 hrs**	**9 hrs**	**12 hrs**	**Mean ±s.e**
31.3	26.7	76.7	83.4	93.4	70.1±17.3bc
15.6	16.7	53.4	73.4	83.4	56.7±14.2b
5.0	16.7	30.0	73.3	80.0	50.0±15.3b
2.5	6.7	13.4	23.4	40.0	20.9±7.5a
1.2	3.3	6.6	13.3	26.6	12.5±4.9a
Mean ± s.e	**14.0±3.5a**	**36.0±12.3b**	**53.4±15.2b**	**64.7±13.6bc**	
Control	**0**	**0**	**0**	**5**	**1.3**

Means of three replicates (± s.e), LSD = 13.56, Means followed by the same letter are not significantly different.

Table 3: The mean percentage mortality effects of different concentrations of **Bifenthrin** on adults of *P. truncatus* from **Benue** at 3 hourly intervals.

Conc. (µg/ml)	**3 hrs**	**6 hrs**	**9 hrs**	**12 hrs**	**Mean ±s.e**
31.3	10.0	36.7	63.4	90.1	50.0±17.2b
15.6	6.7	30.3	60.3	70.3	41.9±14.2b
5.0	6.7	16.7	36.7	66.7	31.7±12.7ab
2.5	3.3	13.3	30.0	36.7	20.8±7.9a
1.2	3.3	6.7	20.0	33.3	15.8±6.7a
Mean ± s.e	**6.0±1.2a**	**20.7±5.3b**	**42.1±8.2c**	**59.4±10.5d**	
Control	**0**	**0**	**0**	**0**	**0**

Means of three replicates (± s.e), LSD = 13.56, Means followed by the same letter are not significantly different.

Table 4 shows that the Ghana strain similarly exhibited increase in percentage mortality with increasing exposure time to Bifenthrin. However, these were not significantly ($P>0.05$; LSD = 15.80) different for the 3rd, 6th and 9th hours. Nevertheless, the response at the 12th hour showed significant increase but it was not different from the response at the 9th hour of exposure. The probit responses of *P. truncatus* strains to the toxicant are presented in Figure 1. This shows that Ibadan strain had LD_{50} value of 10.19 µg/ml and appeared to be the most susceptible, while Benue strain with LD_{50} value of 29.60 µg/ml appeared to be the most tolerant to Bifenthrin. The Ghana and Enugu strains had almost similar levels of response with LD_{50} values of 22.42 µg/ml

Table 4: The mean percentage mortality effects of different concentrations of **Bifenthrin** on adults of *P. truncatus* from **Ghana** at 3 hourly intervals.

Conc. (µg/ml)	**3 hrs**	**6 hrs**	**9 hrs**	**12 hrs**	**Mean ±s.e**
31.3	26.7	50.0	64.1	90.2	57.5±13.3c
15.6	20.0	26.7	50.3	72.1	42.6±11.7b
5.0	16.7	20.0	30.0	47.8	29.1±6.8ab
2.5	13.3	16.6	23.3	23.3	19.1±2.5a
1.2	10.0	10.0	22.0	22.8	16.7±3.3a
Mean ± s.e	**17.3±2.9a**	**24.7±6.5a**	**38.7±8.3ab**	**51.4±13.3b**	
Control	**0**	**0**	**0**	**0**	**0**

Means of three replicates (± s.e), LSD = 13.56, Means followed by the same letter are not significantly different.

and 23.04 µg/ml, respectively. The pyrethriod toxicant showed significant differences ($P<0.05$) in the levels of mortality responses of *P. truncatus* strains from the four different locations.

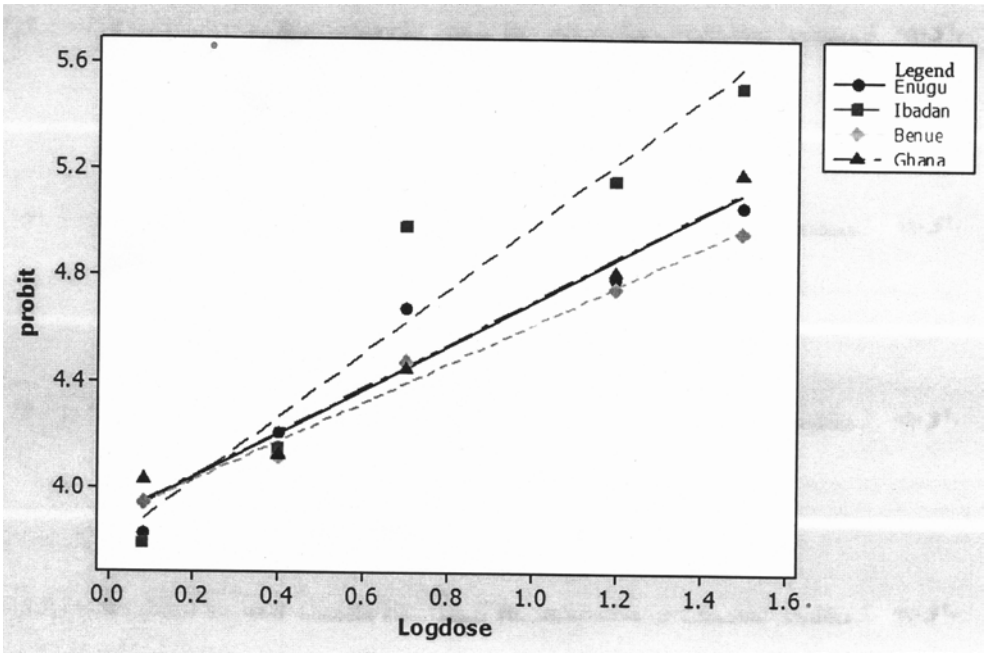

Figure 1: Probit Responses of P. *truncatus* to *Bifenthrin*.

Legends

Enugu strain = $r^2 = 3.885 + 0.819x$; (LD_{50} = 23.04 µg/ml)

Ibadan strain = $r^2 = 3.790 + 1.200x$; (LD_{50} = 10.0 µg/ml)

Benue strain = $r^2 = 3.947 + 0.716x$; (LD_{50} = 29.60 µg/ml)

Ghana = $r^2 = 3.883 + 0.827x$; (LD_{50} = 22.42 µg/ml)

4. Discussion

The laboratory studies conducted to evaluate the effect of exposure time and toxicity of Bifenthrin to adults of the larger grain borer, *Prostephans truncatus* from different geographical locations demonstrated the importance of length of exposure to applied insecticides. In the present studies, mortality increased with increase in the exposure time. After 12 hours of exposure up to 50% of *P. truncatus* from different geographical locations were killed. This showed that with long post-exposure periods, the direct effect of Bifenthrin increased, leading to higher insect mortality. This further, indicates that the product has a long residual activity and could be used as pre-infestation treatment before migrating insects infest the stored products. The author in [19] reported that nearly all storage insects are more active in the dark than in the light and as such most shy away from light. Thus, in the present studies, higher mortalities of *P. truncatus* strains occurred during the later dark hours of exposure. It follows that, timing of application is important in pest management operations, and as suggested by

[20], that a few applications of pesticides would be required if they were timed more accurately in order to reduce selection pressure for resistance.

The present studies therefore, demonstrate the potentials of Bifenthrin pyrethriod in controlling the larger grain borer. The resultant high mortalities of the adult of the insect could be due to highly toxic effect of the toxicant. There was increase in the percentage mortality with increasing concentration as well as the time of exposure progressed. The toxicant contains ketoalchoholic esters of chrysanthemic and pyrethric acids that are highly lipophilic and rapidly penetrate the insects' body cuticle and affect their nervous system [9]. The author [9] further reported that some type II Pyrethroids to which Bifenthrin belongs affect the action of the neuro-transmitter called gamma amino butyric acid (GABA) and that the synergist used with pyrethrins, (piperonyl butoxide, PBO) inhibits mixed-function oxidases (mfo). Other reports in [14] show that Bifenthrin had a high toxic effect on the mortality of the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) and lesser grain borer, *Rhyzopertha dominica* (F.). The toxicant has a broad-spectrum of activity in controlling plant sucking bugs including aphids, weevils, and leaf eating Lepidoptera. Similarly, Deltamethrin, Permethrin, Fenvalerate and Phenothrin performed better than organophospates in causing adult mortality in *P. truncatus* [8]. The results of the present studies agree with these authors that Bifenthrin has high toxic effect and potentials in controlling *P. truncatus.*

In residual treatment, mortality was generally moderate in Bifenthrin treatment when compared with Pyrethroids such as Deltamethrin that were previously reported despite the fact that both are type II pyrethroids. The reasons for this are not clear. One possible explanation may be due to the chemical nature of these compounds and the type of formulations used. Furthermore, the differences in toxicity would depend on the rate of cuticular penetration, solubility in haemolymph and the polarity of the compounds [9], as polar compounds penetrate much faster than the less polar (lipophilic) compounds. Also the toxicity of insecticides depends on the route of transport to the nervous system, the distance to the target site (nervous system), and the permeability of the insect nerve sheath [21] which is more permeable to highly lipo-soluble compounds. There were also dose and time dependent responses of the insects in the present studies. Thus, mortality increased with time in both cases and decreased as concentration decreased.

The *P. truncatus* from Ibadan with the lowest LD_{50} was more susceptible to the toxicant than the other strains from other regions. This occurred in spite of the larger body size of this strain as noted earlier, and this would have required more toxicant to kill. This could be as a result of the physiological constitution of the insects [9]. The other strains with smaller body size and which were tolerant may possess higher concentrations of certain enzymes that confer resistance to insecticides. For example [9] noted that the relative importance of any given metabolic enzyme system may vary from species to species, depending upon the mode of life of the species involved, the degree to which it depends upon that system and the mode of resistance. Thus [22] compared organophosphate-resistant and organophosphate-susceptible strains of a phythophagous mite species, *Tetranychus urticae*, and reported increased levels of mixed functions oxidase. These observations indicate the intra-specific differences in the mechanism of resistance and the differences in the species in the way of utilizing enzyme systems. The *P. truncatus* strain from Benue was more tolerant to the toxicant, possibly it may have acquired tolerance to the toxicant as a result of frequent use and treatment of cassava chips in the area. It should

be noted, however, that much of the cassava chips produced in Nigeria comes from this region and probably treated with insecticides. Thus, it could be suggested that some *P. truncatus* strains used in our present studies may have already developed some degrees of tolerance to Bifenthrin. There is need therefore, to continue monitoring this invasive species for resistance to insecticides.

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