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Insecticide induced changes in haemolymph protein profiles of *Spodoptera frugiperda* (F) (Lepidoptera:Noctuidae)

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ABSTRACT:

Nine insecticides were evaluated for their toxicity (LC_{50}) and 50% lethal times (LT_{50}) against 3rd instar *Spodoptera frugiperda* larvae. Two groups of insecticides were identified based on LC_{50} and LT_{50} values. Bright® 30EC was the most toxic (LC_{50} = 0.0006 µg/g) while Fastac® 5EC was the least toxic (LC_{50} = 0.6046µg/g) among all the insecticides tested. Haemolymph protein changes from insecticide treated larvae were also determined. The total haemolymph protein content in insecticide treated larvae was generally lower than the control. Additionally, the number of protein bands present in electrophoresis gels of insecticide treated larvae was also lower than that of untreated larvae. The implications of these results are discussed.

Keywords:
Spodoptera frugiperda, insecticides, haemolymph proteins, induced changes

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INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (F) (Lepidoptera:Noctuidae) is a serious pest of corn, sorghum and several other grasses in the Neotropics. *S. frugiperda* is an avid flyer which can be found between south-eastern United States to Argentina. A light coloured inverted 'Y' marking is found on the front of its head and its raised, dark shiny spots that occur dorsally on the body distinguishes it from other armyworm species (Sparks, 1979). This pest can cause significant reduction in crop yield and as much as 50% losses in corn in Brazil have been documented (Cruz *et al.,* 1999; Carvalho *et al.*, 2010). Synthetic insecticides are the most commonly used form of control for this pest with a wide variety being utilized (Tavares *et al.,* 2010). Associated with the widespread, frequent use of synthetic insecticides is the development resistance and *S. frugiperda* has been recorded as resistant to several insecticide groups including organophosphates, carbamates and pyrethroids (Yu, 1991).

The effect of synthetic insecticides on the haemolymph proteins of *S. frugiperda* has not been previously studied apart from those involving *Bacillus thuringiensis* (Valdez-Lira *et al.,* 2012). The purpose of this study was to determine the LC_{50} and LT_{50} for nine synthetic insecticides against *S. frugiperda* and to determine insecticide-induced changes in haemolymph proteins in *S. frugiperda* with the aim to better understand the physiological mechanisms for the insecticide induced protein changes.

MATERIALS AND METHODS

Insect culture

An initial stock of *S. frugiperda* larvae was collected on corn (*Zea mays*) from the University of the West Indies Field Station, Trinidad. Larvae were taken back to the laboratory and reared on corn leaves until adult emergence. Adult moths were placed in an insect sleeve cage (30 cm x 30 cm x 30 cm) covered with a fine mesh cloth. Food was supplied *via* a wax paper strip (2cm x 15cm) coated with honey that was mounted to the top of the cage allowing it to hang down. A large bouquet of fresh corn leaves was placed in a glass vial with a cotton wool plug around the rim of the vial to prevent moths from drowning. The bouquet was replaced after the old one had wilted. Cages were checked daily for dead moths and oviposition. Eggs were collected daily from the corn leaves and placed in test tubes for larval emergence. Neonate larvae were transferred to mesh covered plastic containers that had a fresh supply of corn leaves. On the third day after hatching, larvae were placed individually in test tubes with the aid of a small artist's brush (No. 3/0). Neonate larvae were fed with corn leaves until 3rd instar (approximately 10 days) and then used in insecticide bioassays.

Insecticide bioassay

Nine commercial insecticides with different active ingredients were obtained from the University of the West Indies Field Station, Trinidad for use in insecticide bioassays. These insecticides were: Abamectin (abamectin), Boxer® 30EC (etofenprox), Bright® 25EC (carbosulfan), Fastac® 5EC (α-cypermethrin), Flip® 800DF (fipronil), Karate® 5EC (λ-cyhalothrin), Malathion 50 EC (malathion), Neem X® 0.4EC (azadirachtin) and Supertak® 10EC (α – cypermethrin).

A corn (*Zea mays*) leaf dip bioassay was used for each population of *S. frugiperda*. Each bioassay comprised five concentrations for each insecticide (4%, 0.4%, 0.04%, 0.004%, and 0.0004%) and a control. Young corn leaves were cut into 7 cm x 7 cm segments. Each segment was dipped into their respective insecticide concentration solution for 30s, held vertically to permit excess solution to drip off and then placed on paper towel to air dry for 30 minutes. Each treated leaf segment was placed in a 9 cm petri dish with moistened filter paper lining the bottom. *S. frugiperda* 3rd instar larvae were starved for 5 h prior to being placed on

leaves of each petri dish. Five replicates were maintained for the treatment of each insecticide. The control comprised of leaves treated only with distilled water. Petri dish lids were covered with fine gauze to allow for ventilation and prevent fumigant action of the insecticides. Each petri dish was sealed around the edge with clear tape to prevent escape of larvae. Larval mortality was assessed every 2 h for 24 h. Larvae unresponsive to a gentle prod with a toothpick within 5s were regarded as dead. Data were corrected for control mortality using Abbott's (1925) formula. Mortality data were subjected to probit analysis using EPA Probit program Version 1.4.

Protein bioassay

Based on LC_{50} values obtained for each insecticide, 4th instar *S. frugiperda* larvae were subjected to sub-lethal doses on each insecticide for 24 h. Live larvae exposed to a particular insecticide after 24 h were collected and crushed in an Eppendorf tube, centrifuged and the supernatant collected and analyzed for total protein content using Lowry *et al.,* (1951) method and also separated using Polyacrylamide Gel Electrophoresis (PAGE). 7.5% separating gel was prepared from 30% acrylamide-BIS, 10% ammonium persulfate and tetramethylethylenediamine (TEMED). The mixture was then swirled to ensure thorough mixing. The solution was pipetted into Gel Wrap™ Gasket maker and left at room temperature for 45 minutes to polymerize. A 4% stacking gel was prepared using 30% acrylamide-BIS with 10% ammonium persulfate and TEMED and left at room temperature for 45 minutes to polymerize and then refrigerated at 4°C overnight.

Fourth instar *S. frugiperda* larvae were exposed to the lowest concentration (0.0004%) of each insecticide for 24 h before protein extraction took place. Larvae were crushed to a smooth texture in micro-centrifuge tubes containing 100 µl of deionized water. All samples were thoroughly mixed for 5s with the aid of a Vortex Genie 2® machine and centrifuged at 10,000 rpm for two minutes, then gradually increased to 14,000 rpm for 2 minutes. Each sample (35µl) was mixed separately with 35µl sample buffer (1000µl of 50% glycerol, 800µl of running buffer and 200µl of 0.1% bromophenol blue) and 30µl placed in separate lanes together with 20 µl each of the following standards: alpha-lactalbumin (MW= 14.2kDa), carbonic anhydrase (29.0kDa), bovine erythrocytes (45.0kDa), albumin from chicken egg white (66.0kDa) and albumin from bovine serum (66.43kDa). The samples were allowed to run for 1½ h at 180V after which plates were washed with deionized water to remove the gels. Gels were placed into 150cm Pyrex® petri dishes with 100ml of Coomassie blue stain on a Labnet Rocker 25® for 45 minutes to ensure proper and even stain penetration. Gels were then de-stained with 30% methanol: 10% acetic acid for 1h and then rinsed with deionized water (Labban *et al.,* 2012). Bands on the gel were then observed under a fluorescent light and scanned using a UVP Gel Doc-It® 300 imaging system and then analyzed using VisionWorks®LS Analysis Software.

RESULTS AND DISCUSSION

There were two distinct groups of insecticides based on toxicity (LC_{50}) to 3rd instar larvae of *S. frugiperda* (Table 1). The first group comprised Boxer®, Malathion®, Flip®, Bright® and Supertak® among which there were no significant differences ($P>0.05$). The second group comprised Fastac®, Neem-X®, Abamectin® and Karate® among which there were no significant differences ($P>0.05$) but were significantly different ($P>0.05$) from all members of the first group. Bright® 30EC was the most toxic (LC_{50} = 0.0006µg/g) while Fastac® 5EC was the least toxic (LC_{50} = 0.6046µg/g) among all the insecticides evaluated. The active ingredient in both Fastac® 5EC and Supertak®10EC is α-cypermethrin, however their LC_{50} values differed significantly ($P>0.05$) with Supertak®10EC being approximately 62 times more

Table 1. Toxicity of insecticides to 3rd instar *Spodoptera frugiperda* larvae

Insecticide	Probit line	LC_{50} mg/ml (95% CI)*	S.E.	χ^2
Boxer® 30EC	Y = 0.78x + 7.21	0.0014 (0.0002, 0.0089)[a]	2.55	1.94
Malathion® 50EC	Y = 0.88x + 6.72	0.0111 (0.0023, 0.0549)[ad]	2.26	1.38
Flip® 800DF	Y = 1.32x + 8.37	0.0028 (0.0008, 0.0098)[a]	1.91	0.43
Bright® 25EC	Y = 0.60x + 6.95	0.0006 (0.0001, 0.0064)[a]	3.44	0.27
Supertak® 10EC	Y = 0.43x + 5.87	0.0098 (0.0007, 0.1421)[ac]	3.90	1.18
Fastac® 5EC	Y = 0.55x + 5.12	0.6046 (0.0525, 6.9626)[b]	3.48	0.57
Neem-X® 0.4EC	Y = 0.61x + 5.42	0.2052 (0.0255, 1.6487)[b]	2.89	0.22
Abamectin®	Y = 0.46x + 5.17	0.4192 (0.0259, 6.7786)[b]	4.14	0.01
Karate® 5EC	Y = 0.71x + 2.50	0.1339 (0.0230, 0.7781)[bcd]	0.01	0.21

Values followed by the same letter are not significantly different from each other based on Tukey-Kramer Multiple comparisons test

toxic to 3rd instar *S. frugiperda* larvae than Fastac® 5EC (Table 1) and apart from the doubling in concentration, may have been as a result of other components (adjuvants) in the formulation. Mesnage *et al.,* (2014) conducted studies on other pesticides using human cell lines also concluded that adjuvants listed as inert ingredients in pesticides can amplify the toxicity to 1000 -fold.

Among the insecticides tested, Flip® 800DF took the shortest time to cause 50% mortality (LT_{50} = 2.05 h), while Abamectin took the longest (LT_{50} = 18.18 h) which was significantly different (P<0.05) from all the other insecticides tested (Table 2). Abamectin also took the longest to achieve 50% mortality when used against *Spodoptera litura* in Pakistan (Ahmad *et al.,* 2005). Although Bright® 25EC was the most toxic insecticide tested (LC_{50} = 0.0006mg/ml), the 50% lethal time (LT_{50} = 6.63 h) was high, indicating that it would take a population of *S. frugiperda* larvae approximately 6.63 h to achieve 50% mortality at a concentration of 0.0006mg/ml (Tables 1 and 2). However, Flip 800®DF (fipronil) which had a LC_{50} of 0.0028mg/ml was not significantly different (P>0.05) from the LC_{50} of Bright® 25EC but had a LT_{50} = 2.05h (Table 2).

The total haemolymph protein content of larvae treated with seven of the nine insecticides was significantly lower (P<0.05) than that of the control, while Bright® (632.79µg/ml) and Abamectin®

Table 2. Lethal time (LT_{50}) of insecticides to 3rd instar *Spodoptera frugiperda* larvae

Insecticide	Probit line	LT_{50} (h) (95% CI)*	S.E.	χ^2
Boxer® 30EC	Y = 3.36x + 2.82	4.45 (3.20, 6.20)[a]	1.18	0.95
Malathion® 50EC	Y = 5.42x + 2.33	3.12 (2.28, 4.27)[a]	1.17	0.30
Flip® 800DF	Y = 3.77x + 3.83	2.05 (1.28, 3.27)[ac]	0.17	0.17
Bright® 25EC	Y = 2.01x + 3.35	6.63 (3.55, 12.40)[ad]	1.38	0.65
Supertak® 10EC	Y = 2.19x + 3.67	4.04 (2.34, 6.98)[a]	1.32	1.16
Fastac® 5EC	Y = 2.59x + 3.10	5.40 (3.39, 8.62)[ad]	1.27	0.56
Neem-X® 0.4EC	Y = 1.27x + 4.00	6.12 (2.60, 14.41)[a]	1.55	0.69
Abamectin®	Y = 2.26x + 2.15	18.18 (10.59, 31.20)[b]	1.32	0.38
Karate® 5EC	Y = 1.61x + 3.94	4.59 (2.19, 9.64)[a]	1.46	1.02

Values followed by the same letter are not significantly different from each other based on Tukey-Kramer Multiple comparisons test

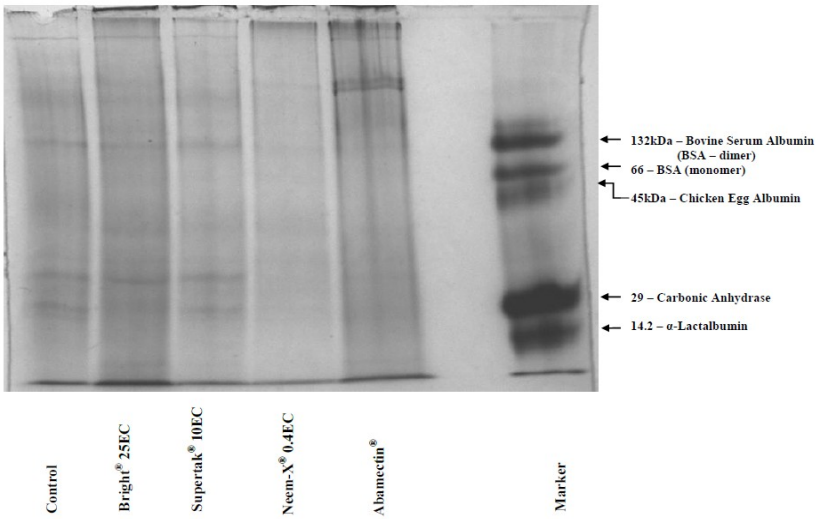

Figure 1 Electrophoresis Gel 1 of haemolymph proteins from *Spodoptera frugiperda* exposed to different insecticides

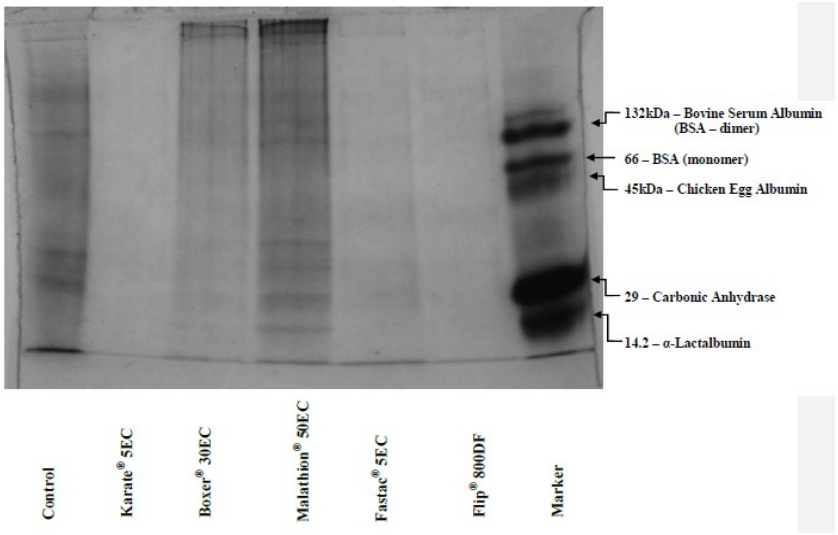

Figure 2 Electrophoresis Gel 2 of haemolymph proteins from *Spodoptera frugiperda* exposed to different insecticides

(617.04g/ml)) were significantly higher ($P<0.05$) than the control. Total haemolymph protein content ranged from 147.46µg/ml (Karate® 5EC) to 632.79µg/ml (Bright® 25EC) (Table 3). Both (Nath *et al.,* 1997 and Usmani and Knowles, 2001) reported that the total protein content in larval haemolymph of insects decreased significantly compared to the control when exposed to organophosphate and pyrethroid insecticides. A similar trend was observed in the present study with larvae of *S. frugiperda.* This haemolymph protein decline may be as a result of increased protein breakdown which may be required to detoxify the components of the

Table 3 Total haemolymph protein content of insecticide treated *Spodoptera frugiperda* 3rd instar larvae

Treatment	Total haemolymph protein content Mean ± SE (µg /ml)*
Control	393.70 ± 2.51 [a]
Karate® 5EC	147.46 ± 3.86 [b]
Boxer® 30EC	244.10 ± 1.97 [c]
Malathion® 50EC	303.51 ± 4.21 [d]
Fastac® 5EC	230.50 ± 1.67 [c]
Flip® 800DF	186.83 ± 1.24 [e]
Bright® 25EC	632.79 ± 2.23 [f]
Supertak® 10EC	352.19 ± 2.62 [g]
Neem-X® 0.4EC	286.33 ± 3.11 [h]
Abamectin®	617.04 ± 1.97 [i]

*Values followed by the same letter are not significantly different from each other based on Tukey test ($P>0.05$)

insecticides tested. As indicated by Nath *et al.,* (1997) the insect may have reduced proteins to their amino acid components to enable their entry to the Tricarboxylic Acid Cycle (TCA) as compensation for stress induced lower energy levels.

The number of protein bands generally decreased in insecticide treated haemolymph compared with the control. The control in Gel 1 had seven bands which ranged from 315.35 µg/ml to 20.13 µg/ml, while Bright, Supertak, Neem X and Abamectin had proteins of molecular weights ranging from (315.35 – 25.39 µg/ml), (315.35 – 24.56 µg/ml), (474.59 – 38.99 µg/ml) and (556.92 – 34.18 µg/ml) respectively (Figure 1). The control in Gel 2 had six bands which ranged from 495.03 µg/ml to 29.46 µg/ml, while Boxer, Malathion, Fastac and Flip had proteins of molecular weights ranging from (407.96 – 13.63 µg/ml), (421.33 – 12.21 µg/ml), (76.30 – 18.95 µg/ml) and (310.18 – 39.23 µg/ml) respectively (Figure 2). Karate insecticide was unusual in that there were no visible protein bands present and may have been as a result of the staining technique.

CONCLUSION

The synthetic insecticides used in the present study caused significant reduction in both total haemolymph protein content and number of proteins in *S. frugiperda* 3rd instar larvae. It is postulated that this may be as a result of the need for amino acids and/or their components to aid in detoxification of these synthetic insecticides via the TCA cycle.

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