

SOME EFFECTS OF AN EC FORMULATION OF AZADIRACHTIN ON IMPORTANT COTTON PESTS IN SENEGAL

George Ekukole*

73-75 Woodbine Avenue
Newark, NJ 07106, USA
ekukoleg@hotmail.com

Accepted: September 28, 2006

Abstract: Laboratory studies were carried out on the effects of an emulsifiable concentrate (EC) formulation of Azadirachtin (AZ) (6 g a.s./l EC) on four major cotton pests in Senegal: *Spodoptera littoralis* Boisduval (*Lepidoptera: Noctuidae*), *Helicoverpa armigera* Hübner (*Lepidoptera: Noctuidae*), *Aphis gossypii* Glover (*Homoptera: Aphididae*) and *Tetranychus urticae* Koch (*Acari: Tetranychidae*). In both *S. littoralis* (SL) and *H. armigera* (HA), larval weight loss was observed 48 h after treatment. Appreciable weight gain was only observed in SL larvae at dosages of 0.05 and 0.1 g a.s./l AZ, 72 h after treatment. In both SL and HA, there was a significant difference ($p < 0.05$) between the control larvae and those treated with 0.1 to 1.5 g a.s./l AZ. At a dosage of 1.5 g a.s./l, AZ caused 60% mortality in SL and 0% mortality in HA, 72h after treatment. The treatment with AZ at dosages of 0.05 and 1.5 g a.s./l resulted in 50 and 30% malformation in SL and HA pupae respectively. The duration of development from the IV instar larva to pupa in both lepidopterans increased progressively with AZ dosage. SL was more susceptible to AZ than HA. No growth disrupting effects were observed in *A. gossypii* (AG) and *T. urticae* (TU) where EC_{50} values of 1.49 and 1.36 g a.s./l AZ respectively were obtained. The implications of the effects of AZ on further field trials against cotton pests are discussed.

Key words: Azadirachtin, growth disruption, mortality, cotton pests, Senegal

INTRODUCTION

The neem tree (*Azadirachta indica* A. Juss.) (*Meliaceae*) originated from India where it has been appreciated for its medicinal and nutritive values for centuries. Extracts from the bark and seeds are now incorporated into soaps, toothpaste and shampoos (Angelini 1989). From India it was introduced to other developing countries. According to the American Academy of Science, the neem tree is about the most useful tree in the arid zones because it is capable of growing even in very poor soils'.

*Formerly: Entomologist – AGROCONSULT 2000 & SENCHIM, Route de Rufisque, Dakar, Senegal

In the West, attention was not paid to the neem until recently. Volonsky (1937) was the first to elucidate the effects of leaf extracts of a close relative of the neem, *Melia azedarach* L., on locusts. Pradhan et al. (1962) showed that extracts from neem seeds diluted in water had a repellent effect on locusts. This pioneering research resulted in more detailed work on the effects of neem extracts and formulations, particularly with regard to the repellent and growth-disrupting properties on several orders of insects, arachnids and nematodes (Jiliani and Malik 1973; Jacobson 1982; Gaaboub and Hayes 1984; Hellpap 1984; Zebitz 1984; Alder and Uebel 1987; Schmutterer 1990; Kaethner 1992; Stoll 1992; PAN and CTA 1995).

Recent research at SENCHIM Dakar (Senegal) and the Department of Analytical Chemistry of the University of Gembloux (Belgium) led to the production of an EC formulation of AZ (6 g a.s./l) (Schiffers et al. 1997). Studies were therefore carried out in the laboratory to elucidate the effects of the said formulation on four important cotton pests in Senegal: *S. littoralis* (leaf-eating lepidopteran), *H. armigera* (bollworm), *A. gossypii* (sap-sucking homopteran) and *T. urticae* (a scarifying spider-mite).

MATERIALS AND METHODS

Rearing of *Spodoptera littoralis* and *Helicoverpa armigera*

Preparation of artificial diet

Specimens of SL and HA used for rearing on artificial diet were obtained as adults from light traps at Tambacounda and Kolda, which are situated at about 500 and 700 km respectively to the south of the capital of Dakar. The following ingredients were used for preparing the diet of both insects (Couilloud and Giret 1978, 1980a, b):

Distilled water	: 1 300 ml
Agar-agar	: 20 g
Sorbic acid	: 2.4 g
Ascorbic acid	: 15 g
Maize flour	: 100 g (modified: Ekukole and Mbaye 1996a)
Beer yeast	: 30 g
Wheat germ	: 30 g (modified: Ekukole and Mbaye 1996a)
Maize oil	: 2 ml
Nipagin (stabilizer)*	: 2 g
Aureomycin (antibiotic)	: 0.1 g

*methyl 4-hydroxybenzoate

Agar-agar was mixed in 1 300 ml of distilled water and heated to 70°C. While stirring, nipagin and sorbic acid were added and the mixture brought to boil and eventually cooled to 60°C. This was followed by addition of ascorbic acid. A mixture of maize flour, wheat germ, yeast and aureomycin was then added and stirred. The diet was finally poured into rectangular polystyrene containers measuring 22 cm x 7.6 cm x 0.5 cm and left to cool at ambient temperature. In order to prevent a deterioration of the diet, the containers were stored in a refrigerator at 10°C.

Rearing on artificial diet

Rearing was done in three types of cages, which were constructed as follows:

- Transparent cylindrical polystyrene cage measuring 22 cm in length and 11.5 cm in diameter with a round hole cut in the lid to make provision for cheese cloth (type **A** cage),
- Transparent polystyrene cage, 11 cm in diameter and 8 cm in height including a lid fitted with fine wire mesh (type **B** cage),
- Transparent polystyrene cage measuring 25 cm × 26 cm × 9 cm including a lid fitted with wire mesh (type **C** cage).

a) *Helicoverpa armigera*

Five pairs of adults (males and females) were introduced into four type A cages. Two polystyrene sampling tubes measuring 5 cm × 2.1 cm, fitted with perforated plastic lids carrying dentist's cotton and containing 10% sucrose solution (10 cubes of sugar dissolved in 250 ml of distilled water and disinfected with two to three drops of 5% sodium hypochlorite solution) were put into each cage to serve as food for the moths. The open ends of the cages were covered with cheese cloth and held in place with the lids for oviposition. The cages were covered with opaque envelopes (29.7 cm × 21 cm) and kept for two days in an insectary maintained at $70 \pm 2\%$ relative humidity and a temperature of $25 \pm 2^\circ\text{C}$. After this period, cheese cloth with eggs was withdrawn (and immediately replaced with fresh cheese cloth), disinfected in 5% sodium hypochlorite solution for 30 sec, air-dried and transferred into type B cages containing cubes (1 cm × 1 cm × 1 cm) of artificial diet. The cages were covered with opaque cardboard boxes (20 cm × 15 cm × 10 cm) to encourage hatching. Lighting was provided from 20-W fluorescent bulbs for 12 h daily. At eclosion, young larvae were transferred into type C cages supplied with cubes of artificial diet. The food supply in these cages was renewed at three to four-day intervals. There were six larval stages which lasted for about 20 days. During the last stage, larvae were transferred into type C cages lined with a peat substrate covered with a sheet of non-absorbent perforated paper to permit the movement of larvae into the peat layer where pupation took place. The pupae were collected, stored at about 15°C in a refrigerator and the rearing process repeated whenever larvae were required for bioassays.

b) *Spodoptera littoralis*

The handling of artificial diet and cages used for rearing SL were similar to those of HA. Instead of cheese cloth, strips of cardboard paper (12 cm × 3.5 cm) were used for oviposition. Type A cages were provided with 8 to 10 pairs of moths and 10% sucrose solution as food in polystyrene vials. Egg batches were collected at two-day intervals, disinfected in 5% sodium hypochlorite solution, air-dried and introduced into type B cages provided with cubes of artificial diet. Occasionally this diet was supplemented with disinfected cotton leaves. The cages were covered with opaque cardboard boxes for hatching which took place in about five days in an insectary maintained under the same conditions as indicated for HA. The young larvae were transferred into type C cages and provided with bars of artificial diet. At about the V instar, the larvae were transferred into type C cages provided with a peat substrate for pupation. Pupae were eventually collected, stored at about 15°C and whenever adults were needed, they were subjected to room temperature to induce emergence.

Rearing of *Aphis gossypii* and *Tetranychus urticae*

Aphis gossypii

Wingless adults were collected from untreated cotton plants (variety: Stam 42) and left in the laboratory for 24 h at ambient temperature for acclimatization. Twenty cotton leaf discs, 8 cm in diameter, were cut from clean and fresh cotton leaves and introduced into a corresponding number of Petri dishes (9 cm inn diameter) provided with moist filter paper (8 cm in diameter). Each dish was artificially infested with one apterous adult. After 24 h, the adults were removed leaving the nymphs. The dishes were held in place with rubber bands and placed in an insectary maintained at temperature and relative humidity of $25\pm 2^{\circ}\text{C}$ and $70\pm 2\%$ respectively (Ekukole 1991, 1996; Ekukole and Mbaye 1996b). Under these conditions the aphid can complete its development in four to five days passing through four instars (Ekukole 1991). Only III instar individuals of the F1 generation were used in the bioassays.

Tetranychus urticae

Samples of two-spotted mite used in *in vitro* tests were obtained from pawpaw leaves in Dakar. The latter were used to artificially infest 5-day old potted groundnut seedlings in out-door cages measuring 50 cm x 50 cm x 50 cm following the protocol of the International Institute of Tropical Agriculture (IITA) Cotonou, Benin (Gnanvossou pers. comm.). Twenty-four hours infestation, the pawpaw leaves were withdrawn and the cages examined to ascertain that the mites had actually moved unto the groundnut leaves. Groundnut was planted serially and infested in the same manner in order to obtain enough individuals for all the bioassays. Only adults bearing the red spots characteristic of the mite were used in the bioassays.

Bioassays with azadirachtin

Preparation of azadirachtin solutions:

A baseline solution (1.5 g a.s./l AZ) was prepared by mixing 250 ml of AZ (6 g a.s./l EC) with one litre of distilled water (250 ml divided by 1000, times 6 g a.s./l). From this solution, serial dilutions were made using a factor of 0.5 to obtain the following concentrations: 0.8, 0.4, 0.2, 0.1 and 0.05 g a.s./l. Distilled water was used for the untreated control.

Tests with *Spodoptera littoralis* and *Helicoverpa armigera*

Seventy newly metamorphosed IV instar larvae were selected from the insectary (average body weight: 125.0 ± 3 mg) and starved for three hours. The larvae were then placed in seven polystyrene cages (13 cm x 9.3 cm x 2.5 cm) each having 10 compartments. Each cage represented a dose and each of the 10 compartments, a replicate. The latter were provided with a cube (1 cm x 1 cm x 1 cm) each of artificial diet which was topically treated with $1\mu\text{l}$ of AZ solution using a Burkard Microapplicator® fitted with a 1 ml syringe. After treatment the cages were returned to the insectary which was maintained at $25\pm 2^{\circ}\text{C}$ and $70\pm 2\%$ relative humidity. The diet in compartments was renewed at 3-day intervals. At 24, 48 and 72 h the larvae were examined for weight gain or loss and thereafter observed daily until emergence of adults. All data collected were subjected to analysis of variance (ANOVA) and the means compared by the least significant difference (LSD).

Test with *Aphis gossypii*

Twenty-one cotton (variety: Stam 42) leaf discs measuring 8 cm in diameter were placed in a corresponding number of petri dishes (9 cm in diameter). The dishes were separated into seven batches, each corresponding to a dosage (including the control) and replicated three times. Each was treated with 2 ml of the corresponding dosage of AZ solution using a Potter Spray Tower calibrated at a pressure of 42 Kpa (6 psi). The leaf discs were air-dried and each dish was infested with 20 third instar AG nymphs which had previously been starved for three hours. Mortality counts were taken at 24, 48 and 72 h. Moribound individuals which barely moved their appendages when probed with a needle were considered dead (Leeper et al. 1988). The data were subjected to probit analysis using an EC_{50} software of the Department of Biometrics of CIRAD (International Centre for Agricultural Research and Development) Montpellier, France.

Test with *Tetranychus urticae*

Infested groundnut leaves were taken from out door cages and brought to the laboratory for acclimatization. The number of individual mites bearing two red spots on each leaf was counted and made up to at least 15 and carefully transferred with a fine brush to clean and fresh cotton leaf discs (8 cm in diameter). Seven batches of leaf discs, replicated three times, were prepared as in the case of AG. Each leaf disc was super-imposed on a thin layer of hydrophilic cotton wool and the set-up mounted on a plastic lid, 8 cm in diameter. The latter was then placed in a petri dish (9 cm in diameter), provided with moist filter paper (8 cm in diameter) to serve as a barrier for escaping mites. Each dish was treated with 2 ml of the appropriate dosage of AZ solution using the Potter Spray Tower as earlier mentioned. Mortality counts were made at 24, 48 and 72 h using the same criterion indicated above. The data were subjected to probit analysis.

RESULTS

Tests with *Spodoptera littoralis* and *Helicoverpa armigera*

The effects of AZ on weight gain, mortality, pupation, adult emergence and duration of development from IV instar larvae to pupae of SL and HL are indicated in Tables 1–4.

Weight gain and loss

Generally weight gain was observed 48 h after treatment among SL larvae. There was a significant difference ($p < 0.05$) in weight gain between the untreated control and the treated larvae at 24, 48 and 72 h after treatment (Table 1). This difference in weight gain was more obvious 72 h after treatment (Table 1). Between the dosages of 1.5 and 0.2 g a.s./l AZ, no appreciable weight gain was observed in SL larvae 72 h after treatment. On the contrary, at dosages of 0.1 and 0.05 g a.s./l AZ, appreciable weight gain was observed after the same period.

Although the same trend was observed in HA larvae, the differences between treatments were comparatively smaller (Table 1) at twenty-four hours observation. There were no significant differences between treatments. However, there was a significant difference ($p < 0.05$) between the untreated and treated larvae at 48 and 72 h

after treatment. The untreated control was at par with 0.05 g a.s./l AZ at 24, 48 and 72 h after treatment (Table 1).

Table 1. Weight gain in IV instar larvae of *Spodoptera littoralis* (SL) and *Helicoverpa armigera* (HA) treated with an EC formulation of Azadirachtin (AZ)

Dosage [g a.s./l]	Weight gain [mg±S.E.] ¹					
	24 h		48 h		72 h	
	SL	HA	SL	HA	SL	HA
1.5	37.0±1.0 b	30.1±1.1	8.1±0.1 c	2.1±0.1 c	9.1±1.0 d	10.1±1.0 c
0.8	32.0±1.3 b	20.3±0.9	9.2±0.1 c	10.2±0.6 c	26.2±2.3 c	10.1±0.1 c
0.4	55.0±2.4 b	30.0±1.4	28.0±1.1 b	30.2±2.1 b	27.2±2.3 c	20.3±2.0 b
0.2	52.0±2.3 b	30.2±1.4	35.1±2.1 b	30.1±2.1 b	34.3±3.3 c	20.4±2.0 b
0.1	40.1±1.3 b	40.1±2.0	34.2±1.5 b	20.1±1.8 b	62.4±3.5 b	30.2±X3.1 b
0.05	60.2±3.3 a	40.0±1.1	35.3±1.0 b	40.1±2.1 ab	79.1±3.3 b	60.1±3.1 a
0.0 ²	99.0±4.3 a	45.0±2.3	102.1±4.3 a	60.5±3.1 a	114.1±4.1 a	70.2±4.1 a

¹Means of 10 larvae

²Untreated control

Original weight of larvae: 125.0±3.0 mg

Means with the same letter in a column are not significantly different at p = 0.05 using LSD

Larval mortality in *Spodoptera littoralis* and *Helicoverpa armigera*

At the highest dosage of 1.5 g a.s./l AZ, 20, 40 and 60% mortality was observed in SL larvae at 24, 48 and 72 h respectively after treatment (Table 2). Only 10% mortality was observed at a dosage of 0.8 g a.s./l AZ, 24 h after treatment. On the other hand, no mortality was recorded among larvae of HA, 72 h after treatment even at the highest dosage.

Table 2. Percentage mortality caused by an EC formulation of Azadirachtin in IV instar larvae of *Spodoptera littoralis* (SL) and *Helicoverpa armigera* (HA) 24, 48 and 72 h after treatment

Dosage [g a.s./l]	% Mortality					
	24 h		48 h		72 h	
	SL	HA	SL	HA	SL	HA
1.5	20.0	0.0	40.0	0.0	60.0	0.0
0.8	10.0	0.0	0.0	0.0	0.0	0.0
0.4	0.0	0.0	0.0	0.0	0.0	0.0
0.2	0.0	0.0	0.0	0.0	0.0	0.0
0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.05	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0

Percentage pupation and adult emergence

In SL, no pupae were obtained from IV instar larvae treated with 1.5 and 0.8 g a.s./l AZ. However, percentage pupation increased from 10% at a dosage of 0.4 g a.s./l AZ to 100% in the untreated control (Table 3). Up to 50% malformation was observed

in pupae derived from IV instar SL larvae treated with only 0.05 g a.s./l AZ (Table 3) whereas in HA, 30% malformed pupae were obtained from larvae treated with the highest dosage of 1.5 g a.s./ AZ (Table 3).

No adults were derived from SL larvae treated with 1.5, 0.8 and 0.4 g a.s./l AZ respectively. The percentage of malformed SL adults increased from 10 to 50 at dosages of between 0.2 and 0.05 g a.s./l AZ. Conversely, only a small percentage of HA adults was abnormal: 30% at the 1.5 g a.s./l AZ and 10% at 0.05 g a.s./l AZ (Table 3). In the untreated control, 90 and 100% respectively of normal HA and SL adults were obtained (Table 3).

Table 3. Percentage pupation and adult emergence in *Spodoptera littoralis* (SL) and *Helicoverpa armigera* (HA) treated with an EC formulation of Azadirachtin

Dosage [g a.s./l]	% Pupation				% Adult emergence			
	Normal		Malformed		Normal		Malformed	
	SL	HA	SL	HA	SL	HA	SL	HA
1.5	0.0	0.0	0.0	30.0	0.0	0.0	0.0	30.0
0.8	0.0	10.0	0.0	20.0	0.0	10.0	0.0	20.0
0.4	0.0	40.0	10.0	0.0	0.0	40.0	10.0	0.0
0.2	50.0	40.0	10.0	0.0	40.0	40.0	10.0	0.0
0.1	60.0	40.0	30.0	0.0	30.0	40.0	30.0	0.0
0.05	90.0	40.0	50.0	10.0	40.0	30.0	50.0	10.0
0.0	100.0	90.0	0.0	0.0	100.0	90.0	0.0	0.0

Duration of development

The duration of development from the IV instar larva to pupa of SL and HA increased significantly ($p < 0.05$) with AZ dosages (Table 4). There was a marked and significant ($p < 0.05$) between the untreated control and AZ-treated larvae of both lepidopterans. No SL pupae were recorded at 1.5 g a.s./l and 0.8 g a.s./l AZ (Table 4).

Table 4. Effect of an EC formulation of Azadirachtin on the duration of development from IV instar larvae to pupae of *Spodoptera littoralis* (SL) and *Helicoverpa armigera* (HA)

Dosage [g a.s./l]	Duration of development [days±S.E.]	
	SL	HA
1.5	*	20.0±1.0 a
0.8	*	20.1±1.0 a
0.4	18.1±1.0 a	18.2±0.8 ab
0.2	14.2±0.9 b	17.1±0.4 ab
0.1	14.0±0.8 b	16.1±0.5 b
0.05	13.1±0.9 b	15.3±0.5 b
0.0	8.3±1.0 c	8.1±0.3 c

*No. pupae recorded

Means with the same letters in columns 2 and 3 are not significantly different at $p = 0.05$ using LSD

Tests with *Aphis gossypii* and *Tetranychus urticae*

Probit analysis of data showed that EC_{50} s expressed as percentage AZ were 1.49 and 1.36 g a.s./l AZ for AG and TU respectively. Azadirachtin appeared to be more toxic to AG than TU (Table 5). Microscopic evaluation of dead individuals of both pests at 24 and 48 h after treatment did not reveal any growth defects or malformations.

Table 5. Effective concentration (EC) values for *Aphis gossypii* (AG) and *Tetranychus urticae* treated with an EC¹ formulation of Azadirachtin (AZ)

Test arthropod	EC_{50} ² (confidence limits)	Slope	Regression
<i>Aphis gossypii</i>	1.49 (0.43–3.83)	0.81	$y = 5.83 + (0.81x)$
<i>Tetranychus urticae</i>	1.36 (0.65–2.86)	1.39	$y = 6.40 + (1.39x)$

¹Emulsifiable concentrate

²Expressed in g a.s./l AZ

DISCUSSION

Natural insecticides from the neem cause mortality in arthropods by growth disruption/regulation or through repellent/antifeedant properties (Schmutterer 1990; Mordue and Blackwell 1993). Most of the published trials were carried out on crude extracts of the neem which is in contrast with this study where an EC formulation of azadirachtin (6 g a.s./l) was tested on artificial diet (for SL and HA) or on actual food substrate (cotton leaves for AG and TU). Such tests have the advantage of being easily reproducible (Morris 1973).

Cotton pests in Senegal and other cotton-growing regions in West and Central Africa can be classified into four major groups: the bollworms (exemplified by *H. armigera*), the leaf-eating worms (*S. littoralis*), the sap-suckers (*A. gossypii*) and mites (*T. urticae*). Any candidate insecticide, be it synthetic or natural, must be tested against these pests in order to identify, among other parameters, its spectrum of activity. The tests reported here have shown that azadirachtin has varying effects on all the pest groups mentioned. At the highest dosage of 1.5 g a.s./l, which corresponds to a field dosage of about 11 g a.s./ha (21/ha), azadirachtin was more efficacious against SL than HA larvae. This may be linked to their different feeding sites and the plant organs consumed in an actual field situation. Since SL is phytophagous, it moves over its food source consuming large amounts whereas HA picks at its food and under field conditions it instinctively attacks flowers, buds and bolls which are situated at specific sites on the reproductive branches. It is therefore possible that because SL consumed more food, particularly at 24 h after treatment, it ultimately ingested more AZ than HA and this might have accounted for the differences in susceptibility observed in the bioassays.

Although the dominant effect against these bollworms was growth-regulating in nature, 48 h after treatment the insects generally lost weight probably due to the repellent/antifeedant effects of AZ. It should be noted that even at the highest dosage of 1.5 g a.s./l, AZ did not prevent both lepidopterans from feeding 72 h after treatment probably because the insects had 'overcome' the barrier created by the bitter taste of AZ. In a field situation, the effects of continuous feeding under high pest pressure could inflict severe damage on the crop. Plants destroyed by SL could easily

be compensated for through the production of new leaves and branches but heavy infestation at the seedling stage could reduce production to nil if replanting were not done in time. With regard to HA, compensation may be difficult because reproductive organs are not easily replaced particularly towards the maturing stage. If used therefore in the field, AZ might not sufficiently control a high infestation of HA and SL at short notice. These findings corroborate those of Angelini (1989) on SL. Perhaps in order to control HA effectively, it would be necessary to use 15–20 g a.s./l of AZ at weekly intervals. However, this dosage may not be economically feasible. Mixtures of AZ and low dosages of a pyrethroid may therefore be cost effective and enhance the efficacy of the product against the noctuids. Ekukole (1997) reported that some microbial products were more effective against bollworms when they were mixed with low dosages of cypermethrin. At weekly intervals, 11 g a.s./ha should be efficacious against a moderate SL infestation but a high population of the leafworm at seedling stage might require the addition of a low dosage of an acaricide.

Unlike the growth-regulating effects of AZ observed in SL and HA, AG and TU were prevented from feeding on treated cotton leaves through the antifeedant/repellent properties of AZ. However, the EC_{50} s for both pests were quite high. AG appeared to be more susceptible than TU possibly because the former is a sap-sucker and the latter a scarifier.

The unique mode of action of AZ appears to be environmentally safer than synthetic pesticides and therefore may lead to new ways of controlling plant pests with natural insecticides (Schmutterer 1990). Azadirachtin should find its place in future IPM programmes on cotton. In the long term, work on the neem and AZ may lead to the synthesis of a chemical product which is more efficacious. Recently a synthetic product was manufactured at the prohibitive cost of USD 1000 per milligramme but this may change as more cost-effective methods of production are being sought (Angelini 1989). This study has shown that AZ used alone cannot effectively control the cotton pest complex in Senegal. Perhaps mixtures of AZ and low dosages of pyrethroids, aphicidal and acaricidal organophosphates could be more effective against these pests.

ACKNOWLEDGEMENTS

I am grateful to Dr. J. W. Everts and W.C. Mullié of the FAO/LOCUSTOX PROJECT in Dakar (Senegal) for their very useful criticisms on the manuscript.

REFERENCES

- Adler V.E., Uebel E.C. 1987. Effects of Margosan-O on six species of cockroaches (Orthoptera: Blattidae & Blattellidae). Proceeding of the Third International Neem Conference, Nairobi, Kenya: 387–392.
- Angelini M. 1989. Activité biologique d'une formulation à base d'Azadirachtine (Margosan-O) à l'égard de *Spodoptera littoralis* (Lepidoptera: Noctuidae). Travail de fin d'études, Faculté des Sciences Agronomiques de Gembloux, Belgique, 92 pp.
- Couilloud R., Giret M. 1978. Améliorations possibles dans la conduite de l'élevage d'*Heliothis armigera*. Note No. 7 – LENI/CIRAD Montpellier, France, 19 pp.
- Couilloud R., Giret M. 1980a. Multiplication d'*Heliothis armigera* Hbn. (Lepidoptera: Noctuidae): améliorations possibles grâce à l'adoption d'une technique d'élevage en groupe des chenilles. Cot. Fib. Trop. 35: 217–224.

- Couilloud R., Giret M. 1980b. *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae): influence de l'espace l'élevage au cours de la vie larvaire sur le rendement en chrysalides et en adultes. Cot. Fib. Trop. 35: 369–376.
- Ekukole G. 1991. Bionomics and control of the cotton aphid, *Aphis gossypii* Glover, in north Cameroon. Ph. D. Thesis, Ahmadu Bello University Zaria, Nigeria, 173 pp.
- Ekukole G., Mbaye B. 1996a. Protocoles pour des tests in vitro des formulations d'Azadirachtine, BPMC et isoprocarbe. Station d'Expérimentation de SENCHIM au CDH Cambérène, Dakar, Senegal, non publié, 5 pp.
- Ekukole G., Mbaye B. 1996b. Rapport sur l'effet in vitro d'Azadirachtine sur *Spodoptera littoralis*, *Helicoverpa armigera*, *Aphis gossypii* et *Tetranychus* sp. Station d'Expérimentation de SENCHIM au CDH Cambérène, Dakar, Senegal, 15 pp.
- Ekukole G. 1996. Prey preference in *Paragus borbonicus* Macquart (Diptera: Syrphidae) larvae and relationship with *Acantholepsis capensis* (Mayr) (Hymenoptera: Formicidae), an ant attending *Aphis gossypii* Glover (Homoptera: Aphididae). J. Afr. Zool. 110: 195–202.
- Ekukole G. 1997. Biological control as a component of IPM on cotton in Sub-Saharan French-Speaking Africa. Proceeding of the CTA/IAR/IIBC Seminar on Integrating Biological Control and Host plant resistance, Addis-Ababa, Ethiopia, 9–14 October 1995, in press.
- Gaaboub I., Hayes D.K. 1984. Biological activity of Azadirachtin, component of the neem tree inhibiting molting in the face fly, *Musca autumnalis* De Geer (Diptera: Muscidae). Environ. Entomol. 13: 808–812.
- Hellpap C. 1984. Effects of neem kernel extracts on the fall armyworm, *Spodoptera frugiperda*. Proceedings of the Second International Neem Conference. Ransischholzhausen, 1983: 353–364.
- Jacobson M. 1982. Neem research in the US dept. of Agric.: chemical, biological and cultural aspects. Proceedings of the First Neem Conference. Rottach-Egern 1980: 33–42.
- Jiliani G., Malik M.M. 1973. Studies on the neem plant as a repellent against stored grain insects. J. Sci. Ind. Res. 16: 251–254.
- Kaethner M. 1992. Fitness reduction and mortality effects of neem-based pesticides on the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). J. Appl. Ent. 113: 456–465.
- Leeper J.R., Raffa K.F., Bruhn J.A. 1988. Analysis of mortality characterization in developing LD values from topical application test results. Trop. Pest Manag. 34: 404–406.
- Mordue A.J., Blackwell A. 1993. Azadirachtin: an update. J. Insect Physiol. 39: 903–924.
- Morris O.N. 1973. Dosage mortality studies with commercial *Bacillus thuringiensis* sprayed in a modified Potter's Tower against some forest insects. J. Invert. Pathol. 22: 108–114.
- PAN, CTA 1995. Pesticides in tropical agriculture: hazards and alternatives. Verlag Josef Margraf, 281 pp.
- Pradhan S., Jotwani M.G., Rai D.K. 1962. The neem seed deterrent to locust. Ind. Fmg. 12: 7–11.
- Schiffers B.C., Dieye A., Ntema P., Dieye B., Ekukole G. 1997. Analyse de l'Azadirachtine par HPLC et développer d'une formulation d'extraits de neem. Med. Fac. Landbouww. Univ. Gent. 62: 225–233.
- Schmutterer H. 1990. Properties and potential of natural insecticides from the neem, *Azadirachta indica*. Ann. Rev. Entomol. 35: 271–297.
- Stoll G. 1992. Natural crop protection: based on local resources in the tropics and sub-tropics. Verlag Josef Margraf, 188 pp.
- Volkonsky M. 1937. Sur l'action acridifuge des extraits de feuilles de *Melia azedrach*. Arch. Inst. Pasteur d'Algérie XV, 3 pp.
- Zebitz C.P.W. 1984. Effect of some crude and azadirachtin-enriched neem (*Azadirachta indica*) seed kernel extracts on the larvae of *Aedes aegyptii*. Entomol. Exp. Appl. 35: 11–16.

POLISH SUMMARY

BADANIA NAD SKUTECZNOŚCIĄ FORMULACJI EC PREPARATU AZADIRACHTIN PRZECIWKO NIEKTÓRYM WAŻNYM SZKODNIKOM BAWĘŁNY W SENEGALU

Prowadzono badania laboratoryjne nad skutecznością formułacji EC preparatu Azadirachtin (AZ) (6 g a.s./l EC) w zwalczaniu 4 głównych szkodników bawełny występujących w Senegalu: *Spodoptera littoralis* Boisduval (*Lepidoptera: Noctuidae*), *Helicoverpa armigera* Hüber (*Lepidoptera: Noctuidae*), *Aphis gossypii* Glover (*Homoptera: Aphididae*) i *Tetranychus urticae* Koch (*Acari: Tetranychidae*). Zarówno w przypadku *S. littoralis* (SL) jak i *H. armigera* (HA) 48 godzin po zabiegu zaobserwowano utratę wagi larw. Wykrywalne zwiększenie wagi obserwowano jedynie w przypadku larw SL i dawek 0,05 oraz 0,1 g a.s./l AZ, 42 godziny po zabiegu. W przypadku SL oraz HA stwierdzono istotną różnicę ($p < 0,05$) pomiędzy larwami z kombinacji kontrolnej i larwami traktowanymi dawkami 0,1 do 1,5 g a.s./l AZ. Preparat AZ użyty w dawce 1,5 g a.s./l spowodował po 42 godzinach po zabiegu 60% śmiertelności SL i 0% śmiertelności HA.

Traktowanie preparatem AZ użytym w dawkach 0,01 i 1,5 g a.i./l spowodowało odpowiednio 50 i 30% zniekształceń poczwarek SL i HA. Czas trwania rozwoju IV stadium larwalnego do stadium poczwarki, zarówno w przypadku SL jak i HA (*Lepidoptera*) wzrastał wraz ze zwiększaniem dawki preparatu. Gatunek *S. littoralis* (SL) był bardziej wrażliwy na działanie AZ niż gatunek *H. armigera* (HA). Nie obserwowano przerwania rozwoju *Aphis gossypii* (AG) i *Tetranychus urticae* (TU), a wartości EC_{50} wynosiły odpowiednio 1,49 i 1,36 g s.a./l AZ. Omówiono uzyskane wyniki pod kątem wykorzystania ich w przyszłych doświadczeniach polowych nad zwalczaniem szkodników bawełny.