Insecticidal properties of an alkaloid from *Alstonia boonei* De Wild

Oigiangbe Osawe Nathaniel[1], Igbinosa Igho Benjamin[1] and Tamo Manuele[2]

ABSTRACT

The insecticidal properties of the alkaloid obtained from *Alstonia boonei* De Wild stem bark was tested against the maize stem borer, *Sesamia calamistis* Hampson in a laboratory bioassay. Less than one day old (less than 24 h after hatching) *S. calamistis* larvae were reared on artificial diet treated with the alkaloid at a rate of 0.00125 %, 0.00100 %; 0.00050 %, 0.00025 %, and 0.00000 % (Control). There was no significant difference between the treatments in the survival of the larvae (range 66.67 % to 86.67 %). at 15 days after introduction (DAI). There were significant differences ($P < 0.05$) in the wet and dry weights of these larvae, which ranged from 25.50 mg in the diet containing 0.00125 % of the alkaloid to 36.30 mg in the 0.00050 % and 5.90 mg in the 0.00125 % to 8.80 mg in the 0.00025 %, respectively. The natural response rate *C*, (OPTC) for the larval survival at 15 DAI was 0.25. Percentage pupation was significantly higher in the Control (80 %) compared with the rest treatments (range 40 – 53.33 %). Pupal weight was significantly higher in the Control (213.67 mg) compared with the 0.00100 % treatment (137.83 mg). The natural response rate *C*, (OPTC) for pupation was 0.42 with an LD_{50} of 0.02662 %. While all the pupae from the Control successfully emerged into the adults, percentage adult emergence for pupae from other treatments ranged from 0.00 to 35.00 %. Various stages of unsuccessful adult emergence were observed in the treatments containing the alkaloid. Total developmental period ranged from 45 days in the 0.00025 % to 58 days in the 0.00125 %. These results show a very high level of insecticidal property of *A. boonei* stem bark alkaloid against *S. calamistis*. We believe that this alkaloid can play a major role in the management of this insect and similar insect pests of tropical crops.

Key Words: *Alstonia boonei*, *Sesamia calamistis*, alkaloid, insecticidal

INTRODUCTION

Alstonia boonei De Wild is a large evergreen tree belonging to the family Apocynaceae. It is distributed throughout the tropics and rain forests of West and Central Africa where the roots, leaves, stem bark, latex, flowers and fruits are used extensively for medicinal purposes (Oze et al., 2007). Several workers have reported that alcoholic or aqueous preparations from parts of the plant, especially the stem bark, are effective in the treatment of febrile illness, jaundice, rheumatism, malaria, fever, intestinal helminthes, and hypertension as well as an antivenom against snake bite (Ojewole, 1984; Asuzu and Onaga, 1991; Olajide *et al.*, 2000; Terahima, 2003; Betti, 2004; Abel and Busia, 2005). Fasola and Egunyomi (2005) reported that the major phytochemicals in the stem bark of *A. boonei* are saponins, alkaloids, tannins, and cardiac glycosides. This bark is known to contain some chemical compounds of the indole alkaloid group namely alstonine, porphine and alstonidine as well as triterpenoids (Phillipson *et al.*, 1987; Anonymous 1992; 2001). Plant alkaloids are a major source of bio-insecticides especially since the discovery of Azadirachtin from the neem tree, *Azadirachta indica* A. Juss (Maala *et al.*, 2000; James *et al.*, 2003; Bruce *et al.*, 2004). Facknath and Lalljee (2008) reported that alkaloids and tannins from *Ayapana triplinervis* (Vahl) R. M. King & H. Rob exhibited feeding deterrence against *Plutella xylostella* L. and *Crocidolomia binotalis* Zeller. The bioactivity of other species of the family Apocynaceae against different insect species have been reported by McLaughlin *et al.* (1980), Raju *et al.* (1990), El-Lakwah *et al.* (1996) and Jeong *et al.* (2001). The aqueous and methanol extracts of the leaf and stem bark of *A. boonei* have been found to be bioactive against *Maruca vitrata* Fabricius and *Sesamia calamistis* Hampson (Oigiangbe *et al.*, 2007a, b). This paper reports on the bioactivity of *A. boonei* alkaloids against the African pink borer, *Sesamia calamistis* Hampson which is a major pest of maize (*Zea mays* L.) and other cereal crops in West and Central Africa (Bosque-Perez and Mareck,

1990 and 1991; Oigiangbe *et al.*, 1997; Kalule *et al.*, 1997; Banwo, 2002; Obhiokhenan *et al.* 2002).

MATERIALS AND METHODS

Extraction of alkaloid

Alstonia boonei stem bark was collected from mature trees (20 – 35 m in height), dried in the sun and ground to powder using an electric blender (SuperMaster®, Model SMB 2977, Japan). About 250 g of the powder was extracted in the cold with about 300 ml of 50 % aqueous methanol in 1 liter Schott Duran bottles for 72 h. The extracts were filtered through a baft cloth. The extraction process was repeated twice for maximum yield. The extracts were bulked together in a 2.5 l Winchester bottle and basified with sufficient amount of anhydrous sodium hydroxide (NaOH) pellets until the alkaloid precipitation became very visible (Anonymous, 2000). The free base alkaloid was removed from the solution by adding 1 liter of Chloroform into which it dissolved. The alkaloid was obtained by evaporating the Chloroform in a Gallenkamp Hotbox oven at 40 °C to dryness and kept in sealed glass vials until needed.

Bioassay

The bioassay was done by incorporating the alkaloid into artificial diet for *S. calamistis* at a rate of T1 - 0.00000 % (Control), T2- 0.00025 %, T3- 0.00050 %, T4- 0.00100 % and T5- 0.00125 % (w/w). The treatments were dispensed into wells of a plastic tray (Bioserv®) and allowed to cool down and solidify under a microflow workstation. *Sesamia calamistis* larvae were obtained from the colony in the Insect Rearing Unit the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. They were maintained on the artificial diet for two generations. Two larvae (< 24 h old) were introduced into each well of the assay tray containing the treated and control diets. Each plastic had twenty four wells and represented a treatment. The plastic trays were sealed with a perforated nylon sheet to allow for ventilation. There were three replicates. The treat ments were arranged randomly in trays in the laboratory maintained at a temperature of 26 ± 2 °C, 80 ± 5 % relative humidity and 14 h : 10 h (light : dark) illumination. Larval survival and weight were recorded at 15 days after introdu ction (DAI) from twelve wells per treatment. The fresh weight of the larvae was taken immediately after removal from the diet while the dry weight was taken after drying in the oven for 14 days at 40°C. The natural response rate *C* (OPTC) and LD_{50} were calculated at15 DAI and after pupation using probit analysis while data on survival and weight were analyzed using the analysis of variance (ANOVA) (SAS System for Windows, Version 9.1.3). Means were separated with the Fisher's Least Significant Difference (LSD).

RESULTS

There was no significant difference between the treatments in the percentage survival of the *S. calamistis* larvae (Figure 1) at 15 DAI. Plate 1 (A and B) shows the natural response rate, (*C*), or curve of the larvae. The LD_{50} for larval survival could not be established because all the treatments allowed over 50 % of the larvae to survive. The wet and dry weights of the larvae reared on the diet containing the highest concentration of the alkaloid were significantly lower ($P < 0.05$) than those reared on some of the other treatments (Figures 2 and 3). The alkaloid had multiple effects on the size and weight of the pupae (Figure 4), with some malformed, indicating growth disruption. The percentage pupation was significantly higher ($P < 0.05$) in the Control compared with the other treatments (Figure 5). The natural response rate, OPTC, and LD_{50} at the pupal stage are shown in Plate 2 (A and B). The percentage adult emergence was significantly higher in the Control compared with the other treatments (Figure 6). Adult size and weight were also significantly ($P < 0.05$) affected by the alkaloid (Figure 7, Plate 3 A). Emergence was disrupted or incomplete in some cases (Plate 3 B).

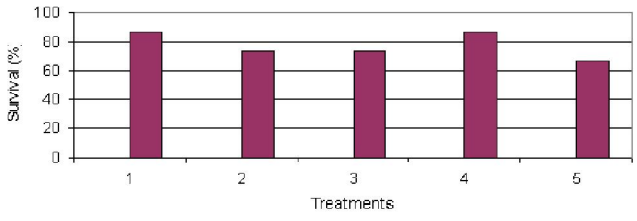

Figure 1. Percentage survival of *S. calamistis* larvae exposed to different concentrations of *A. boonei* stem bark alkaloid at 15 DAI.

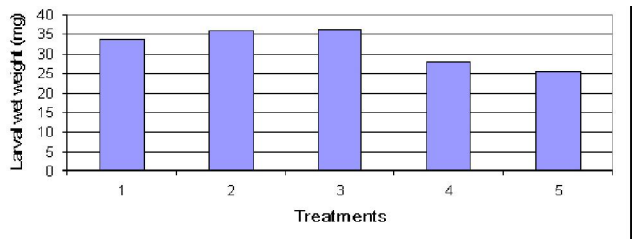

Figure 2. Mean larval wet weight (mg) of *S. calamistis* larvae exposed to different concentrations of *A. boonei* stem bark alkaloid at 15 DAI

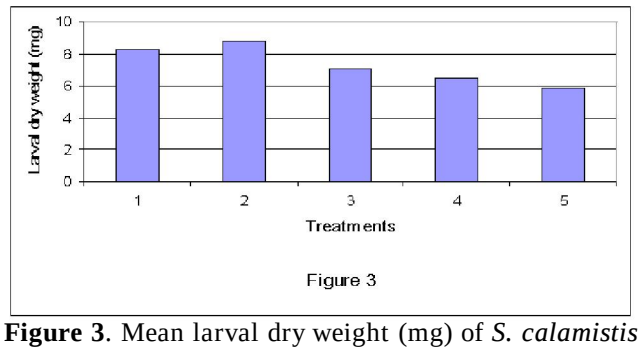

Figure 3. Mean larval dry weight (mg) of *S. calamistis* exposed to *A. boonei* stem bark alkaloid at 15 DAI.

Figure 4. Mean pupal wet weights (mg) of *S. calamistis* larvae exposed to different concentrations of *A. boonei* stem bark alkaloid.

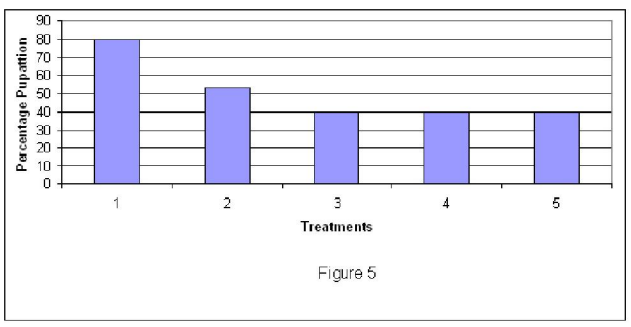

Figure 5. Percentage pupation of *S. calamistis* exposed to different concentrations of *A. boonei* stem bark alkaloid.

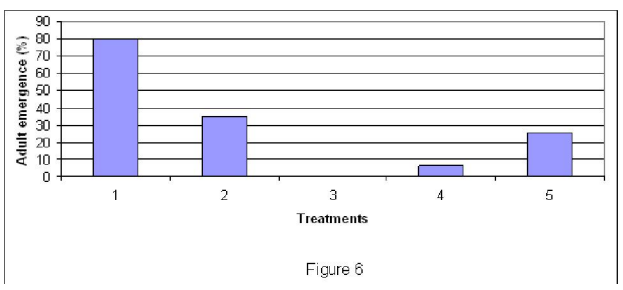

Figure 6. Percentage adult emergence of *S. calamistis* exposed to different concentrations of *A. boonei* alkaloid.

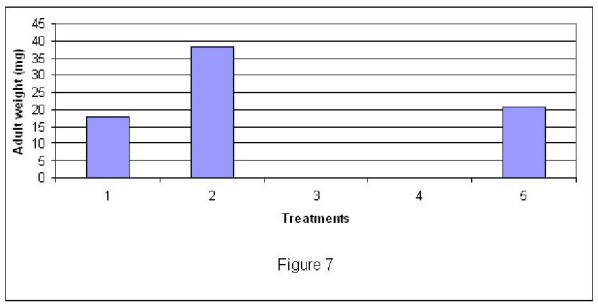

Figure 7. Mean adult weight (mg) of *S. calamistis* reared on different concentrations of *A. boonei* alkaloid.

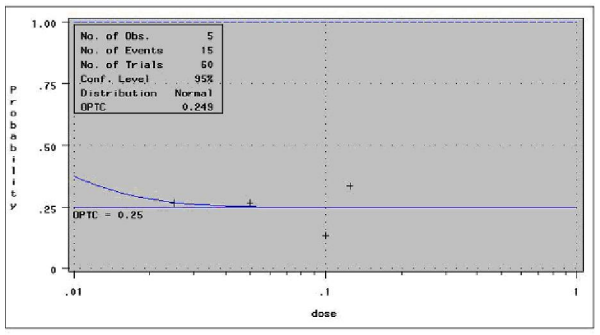

Plate 1A

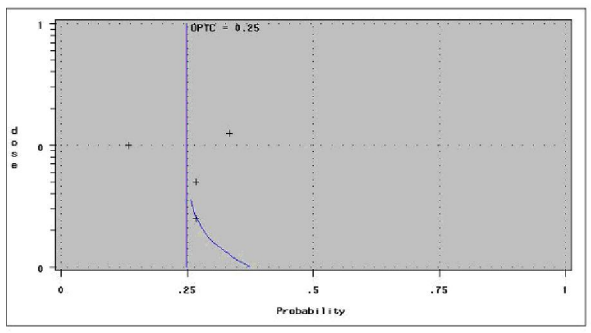

Plate 1B

Plate 1 (A and B). Natural response curves of *S. calamistis* larvae exposed to different concentrations of *A. boonei* alkaloid.

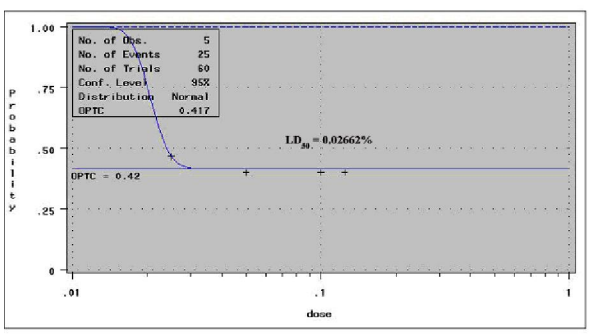

Plate 2A

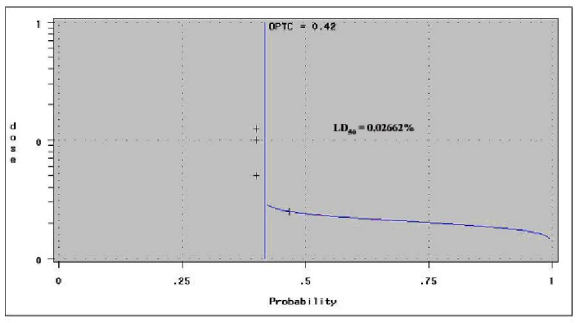

Plate 2B

Plate 2 (A and B). Natural response curves of pupae from *S. calamistis* larvae exposed to different concentrations of *A. boonei* alkaloid.

Plate 3A

Plate 3B

Plate 3 (A and B). Effects of *A, boonei* alkaloid on the growth and development of *S. calamistis.*

DISCUSSION

The results of this study show that *A. boonei* stem bark alkaloid is insecticidal against *S. calamistis*. The insecticidal effects included reduced larval survival and pupation as well as growth disruption. The growth disruption effects were the inability of some of the larvae to successfully molt into the pupal stage or some of the pupae into the adult stage. That the LD_{50} of the alkaloid was 0.02662 % at the pupal stage shows its great potential as an effective botanical insecticide for the management of *S. calamistis*. This is lower than the 0.05 % active ingredient in most commercial pyrethroid products used in insect pests control. Natural products that are bioactive against insects have been recognized as control agents for insect pests, but more especially as they give insights into the development of some insecticides (Russel, 1977). Among the most important of the natural alkaloids used in insect control are nicotine and the related compound nornicotine, while veretrine and ryanodin are of less importance (Balandrin *et al.*, 1985). Members of the dogbane family, Apocynaceae, are known to contain the indole alkaloids (Phillipson *et al.*, 1987; Trindade *et al.*, 2008). Insecticidal properties have been reported for *Aspidosperma pyrifolium* Mart., a member of this family, against the diamondback moth, *P. xylostella* (Torres *et al.*, 2001 and 2006; Trindade *et al.*, 2008). Trindade *et al.* (2008) also reported that a large number of *P. xylostella* larvae died when fed on leaf discs treated with *A. pyrifolium* alkaloids. The death resulted from the inability of the larvae to fully cast off the old exoskeleton, which typically remained linked to the posterior part of the abdomen. Murdue-Luntz and Nisbet (2000) also observed similar symptoms in insect larvae exposed to different concentrations of azadirachtin, an alkaloid from the neem tree, *A. indica*. These authors attributed the symptoms to the reduction in the concentration of ecdysone or to its delayed release into the circulatory fluid. We conclude that *A. boonei* stem bark alkaloid has multiple insecticidal effects against *S. calamistis* with a great potential as a candidate in the search for new active compounds for the management of this and related insect pests of tropical crops.

REFERENCES

Abel, C., Busia, K., 2005. An Exploratory Ethnobotanical Study of the Practice of Herbal Medicine by the Akan Peoples of Ghana (Ethnobotanical Study: Herbal Medicine in Ghana). *Alternative Medicine Review*, 42 **P.**

Anonymous, 1992. Monograph of *Alstonia* Bark. **In:** *Ghana Herbal Pharmacopoeia*. The Advent Press, Accra Ghana, 10-12 **P.**

Anonymous, 2000. Acid Base Extraction @Every thing2.com

Anonymous, 2001. *Earthnotes Herb Library*. Ernestinar Parziale CH.

Asuzu, U. and Anaga, A. O. 1991. Pharmacological screening of the aqueous extract of *Alstonia boonei* stem bark. *Filoterapia,* **63:** 411-417.

Balandrin, M. F., Kloeke, J. A., Wurtele, E. S. and Bollinger W. H. 1985. Natural plant chemicals: source of industrial and medicinal materials. *Science,* **228**: 1154-1160.

Banwo O. O. 2002: Management of major insect pests of rice in Tanzania. *Plant Protection Science,* **38**: 108–113.

Betti, J. L., 2004. An ethnobotanical study of medicinal plantsa mong the Baka Pygmies in the dja biosphere reserve, Cameroon. *African Study Monographs*, **25**(1):1-27.

Bosque-Perez, N. A., Mareck, Z. T., 1990. Distribution and composition of Lepidopterous maize borers in southern Nigeria. *Bulletin of Entomological Research*, **80**: 363-368.

Bosque-Perez, N. A. and J. H. Mareck 1991. Effect of the stem borer *Eldana saccharina* (Lepidoptera: Pyralidae) on the yield of maize. *Bulletin of Entomological Research,* **81:** 243-247

Bruce, Y. A., Gounou, S., Chabi-Olaye, A., Smith, H., Schulthess, F., 2004. The effect of neem (*Azadirachta indica* A Juss) oil on oviposition, development and reproductive potentials of S*esamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Eldana saccharina* Walker (Lepidoptera:Pyralidae). *Agricultural and Forest Entomology*, **6**(3): 223-232.

Deutsch, H. F., Everson, M. A., Drescher, P., Sparwasser, C. Madsen, P. O. 1994. Isolation and biological activity of aspidospermine and quebrachamine from an *Aspidosperma* tree source. *Journal of Pharmaceutical and Biomedical Analysis,* **12**: 1283-1287.

El-Lakwah, F. A., Hamed, M. S., Abdel-Latif, A. M., 1996. Effectiveness of *Lantana camera* and *Nerium oleander* extracts alone and in mixtures with two insecticides against *Rhizopertha dominica* (F.). *Annals of Agricultural Science Moshtohor*, **34**:1879-1905.

Facknath, S. and Lalljee, B. 2008. Study of various extracts of *Ayapana triplinervis* for their potential in controlling three insect pests of horticultural crops. *Tropicultura* **26**(2): 119-124.

Fasola, T. R., Egunyomi, A., 2005. Nigerian usage of bark in phytomedicine. *Ethnobotany Research and Applications*, **3**: 73-77.

Huang, Q., Kong Y., Liu, M., Feng, J. and Liu Y. 2008. Effect of oxadiazolyl 3(2H)-pyridazinone on the larval growth and digestive physiology of the armyworm, *Pseudaletia separata. Journal of Insect Science,* **8**(19), 7 **P**.

James, B., Caulibaly, O., Gbaguidi, B., 2003. New solutions for cowpea production in Africa. *Pesticide News,* **61**: 12-13.

Jeong, S. E., Lee, Y., Hwang, J. H., Knipple, D. C., 2001. Effects of the sap of the common oleander *Nerium indicum* (Apocyanaceae) on male fertility and spermatogenesis in the oriental tobacco budworm *Helicoverpa assulta* (Lepidoptera: Noctuidae). *The Journal of Experimenta Biology*, **204**: 3935-3942.

Kalule, T., Ogenga-Latigo, M. W. and Okoth, V. O. A. 1997. Seasonal fluctuations and damage of lepidopteran stemborers of maize in a major agroecozone of Uganda. *African Crop Science Journal*, **5**(4): 385-393.

Maala, E., Kiiya, W., Omollo, P., Omondi, P., 2000. Participatory Evaluation of the Effectiveness of Locally Known Plant Extracts and Insecticides In Controlling Maize Stalk Borer. http://www.kari.org/Legume_Project/Legume2 Conf_2000/44.pdf

McLaughlin, J. L., Freedman, B., Powel, S., Smith, C. R. Jr. 1980. Neriifolin and 22 acetyl neriifolin insecticidal and cytotoxic agents of *Thevitia thevetoides* seeds. *Journal of Economic Entomology*, **73**:398-402.

Mordue-Luntz, A. J., Nisbet, A. J. 2000. Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. *Anais da Sociedade Entomológica do Brasil* **29**: 615-632.

Obhiokhenan, A. A., Usua., E. J. and Umoetuk, S. B. A. 2002. Distribution of Lepidopterous stemborers of maize (*Zea mays* L.) in ecological zones of Cross River State of Nigeria. *Global Journal of Agricultural Science* **1**(2): 89-92.

Oigiangbe, O. N., Ivbijaro, M. F., Ewete, F. K. and Mutsaers, H. J. W. 1997. Incidence and damage cursed by maize stemborers on farmers' fields in south western Nigeria. *African Crop Science Journal*, **5**(3): 295-302

Oigiangbe, O. N., Igbinosa, I. B., Tamo, M., 2007a. Bio activity of extracts of *Alstonia boonei* (Apocynaceae) De Wild Stem Bark against *Maruca vitrata* (Lepidoptera: Pyralidae) Fabricius. *Advances in Science and Technology*, **1**(1): 67-70.

Oigiangbe, O. N., Igbinosa, I. B. and Tamo, M. 2007b. Insecti cidal activity of the medicinal plant, Alstonia boonei De Wild, against *Sesamia calamistis* Hampson. *Journal of Zhejiang University Science B*, **8**(10): 752-755.

Ojewole, J. A. O. 1984. Studies on pharmacology of echitamine, an alkaloid from the stem bark of *Alstonia boonei. International Journal of Crude Drug Research* **22**: 121-143.

Olajide, A. O., Awe, S. O., Makinde, J. M., Ekhelar, A. I., Olusola, A., Morebise, O. and Okpako, D. T. 2000. Studies on the anti-inflammatory, antipyretic and analgesic properties of Alstonia boonei stem bark. *Journal of Ethnopharmacology,* **71**: 179-186.

Oze, G. O., Nwanjo, H. U., Onyeze, G. O. 2007. Nephro toxicity caused by the extract of *Alstonia boonei* (De Wild) stem bark in Guinea pigs. *The Internet Journal of Nutrition and Wellness*, **3**(2): 1-7.

Phillipson, J. D., O'Neill, M. J., Wright, C. W., Bray, D. H., Warhurst, D. C. 1987. Plants as Sources of Antimalarial and Amoebicidal Compounds. *In*: *Medicinal and Poisonous Plants of the Tropics: Proceedings of Symposium 5~35 of the 14th International Botanical Congress*. Berlin July 24 - Aug.1, 70-78 **PP**.

Raju, M., Thakur, K., Maruthi, R. G. 1990. Sterilizing activity of the flower extract of *Thevitia neriifolia* on the male fifth instar nymph of red cotton bug, *Dysdercus similis* F. (Heteroptera: Pyrrhocoridae). *Journal of Animal Morphology and Physiology*, **37**:77-78.

Russell, G. B. 1977. Plant Chemicals affecting insect development. *The New Zealand Entomologist,* **6**(3): 229-234.

Terashima, H., 2003. Names, use and attributes of plants and animals among the Ituri forest foragers: A comparative ethnobotanical and ethnozoological study. *African Study Monographs*, **28**(Suppl.):7-24.

Torres, A. L., Barros, R., Oliveira, J. V. 2001. Efeito de plantas no desenvolvimento de *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Neotropical Entomology,* **30**: 151-156.

Torres, A. L., Boiça Júnior, A. L., Medeiros, C. A. M.; Barros, R. 2006. Efeito de extratos aquosos de *Azadirachta indica, Melia azedarach* e *Aspidosperma pyrifolium* no desenvolvimento e oviposição de *Plutella xylostella*. *Bragantia*, **65**: 447-457.

Trindade, R. C. P., da Silva, P. P., de Araújo-Júnior, J. X., I. S. de Lima; J. E. de Paula A. E. G. Sant'Ana 2008. Mortality of *Plutella xylostella* larvae treated with *Aspidosperma pyrifolium* ethanol extracts. *Pesquisa Agropecuária Brasileira,* **43**(12): 6 **P**.

Oigiangbe Osawe Nathaniel[1], Igbinosa Igho Benjamin[1] and Tamo Manuele[2]

[1]Department of Zoology, Ambrose Alli University, Ekpoma, Edo State, Nigeria, Phone : +234(0)8035755679, Email : osawe_oigiangbe @hotmail.com.

[2]International Institute of Tropical Agriculture (IITA), Benin Station, Cotonou, Benin Republic.