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Bioactivity of *Alstonia boonei* De Wild leaf alkaloid on the growth and development of *Maruca vitrata* Fab.

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ABSTRACT

The bioactivity of *Alstonia boonei* De Wild leaf alkaloid was tested under laboratory bioassay against the legume pod borer, *Maruca vitrata* Fabricius. The alkaloid was incorporated into standard *M. vitrata* artificial diet at five doses [0.00 % (Control), 0.02 %, 0.05 %, 0.10 % and 0.20 %], and fed to the newly emerged first instar *M. vitrata* larvae in plastic wells under laboratory conditions. Significant differences ($P < 0.05$) were recorded in the larval survival, wet and dry weights of the larvae at ten days after infestation (DAI). Mean larval survival ranged from 34.78 to 65.00 percent in the treatments with the alkaloid compared with 91.67 percent in the control. At 15 DAI, all the survivors in the control had pupated normally compared with the 4.34 to 35.00 percent pupation (with various levels of deformity) in the treatments with the alkaloid. The mean percentage adult emergence was also significantly different in the control (83.33 %) compared with the other treatments (range 0.00 to 10.00 %). These results indicate a high level of bioactivity of *A. boonei* leaf alkaloid against *M. vitrata*. It is concluded that the alkaloid has a great potential in the search for bioactive compounds for the management of the insect.

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INTRODUCTION

The legume pod borer, *Mauca vitrata* Fabricius, is one of the major insect pests of cowpea in Africa (Jackai and Daoust, 1986; Timko and Singh, 2008). The larvae feed extensively on the flower buds, flowers, pods and young stems causing up to 80 % yield loss (Sharma, 1998; Nampala *et al*., 2002; Dannon *et al*., 2010). Losses due to *M. vitrata* have been estimated at US$ 30 million annually (Rout and Sardar, 2011). The use of synthetic insecticides is the most reliable method of controlling this insect pest as no profitable cowpea crop can be successfully grown without at least two insecticide sprays (Jackai and Adalla, 1997). The cost, availability and human/environmental hazards caused by these insecticides have made the search for alternative control measures inevitable and urgent.

Attention has been directed towards botanical insecticides as many antifeedants, repellents and growth retardants or disruptive compounds have been isolated from plants. Antifeedants are important in plant protection because they provide a first line of defense against notorious insect pests (Arivoli and Tennyson, 2013). Koul (2005) reported that the most effective insect feeding inhibitors are terpenoids, alkaloids, saponins and polyphenols. The discovery of new antifeedants has been described as a potential method for the development of ecologically safe pesticides (Wheeler *et al*., 2001).

Alstonia boonei De Wild (Apocyanaceae) grows into a giant tree in most of the lowland and high rain forest of tropical Africa and is well known to all the traditional healers practising along the West African coast (Moronkola and Kunle, 2012; Adotey *et al*., 2012). Chemical compounds isolated from *A. boonei* include alkaloids, tannins, saponins, flavonoids, iridoids, cardiac glycosides and triterpenoids (Ayiku, 1992; Kucero *et al*., 1992; Fasola and Egunyomi, 2005; Afolabi *et al*., 2007). Six alkaloids namely: echitamine (1), echitamidine, voacangines, akuammidine, Nα-formylechitamidine and Nα-formyl-12-methoxyechitamidine have been isolated from the stem bark of *A. boonei* (Adotey *et al.*, 2012). Extracts of *A. boonei* leaf showed insecticidal activity against *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) previously (Oigiangbe *et al.*, 2007).

This paper reports the bioactivity of alkaloid isolated from fresh leaves of *A. boonei* against *M. vitrata* under a laboratory bioassay.

MATERIALS AND METHODS

Fresh leaves of *A. boonei* were collected from a mature (over twenty years old) tree, taken to the laboratory, rinsed in tap water and blended into a paste with an electric blender using 50 % aqueous methanol as solvent.

The alkaloid was extracted according the method of Oigiangbe *et al*. (2007). Briefly, the *A. boonei* leaf paste was soaked in sufficient 50 % methanol for two nights, filtered through a muslin cloth, and to the filtrate was added enough pellets of anhydrous sodium hydroxide (NaOH) to make the alkaloid insoluble in the solvent. An equal volume of chloroform was added to the solution into which the alkaloid dissolved. The chloroform portion was removed from the mixture with a separating funnel and concentrated in a rotary evaporator to obtain the alkaloid. The alkaloid was kept in a sealed vial in the refrigerator until needed. The alkaloid was incorporated into standard *M. vitrata* diet (Jackai and Raulston, 1988) at a rate of 0.00 % (control), 0.02 %, 0.05 %, 0.10 % and 0.20 % and fed to newly emerged (> 24 h old) *M. vitrata* larvae in plastic wells.

There were three replicates and the experiment was arranged in trays on a laboratory shelve at a temperature of 25 ± 2^{o}C and relative humidity of 75 ± 5 % in a completely randomized design. Larval survival, wet and dry weights were recorded at 10 days after infestation (DAI). Survival and life forms were recorded at 15 DAI. Percentage adult emergence was also noted. Data was analyzed using the analysis of variance (ANOVA) on SAS Release 9.2 (2013). Means were separated using the Fischer's Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Figure 1 shows the percentage larval survival at 10 and 15 DAI. Treatments with *A. boonei* alkaloid significantly reduced the survival of the larvae compared with the control. The alkaloid affected the larval weight in a dose-dependent manner. The wet and dry weights of the larvae at 10 DAI are shown in Figure 2.

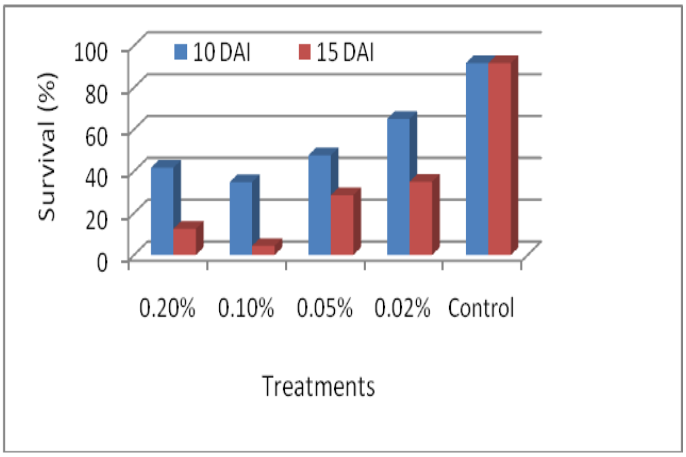

Figure 1. Mean percentage survival of *M. vitrata* larvae fed on artificial diet mixed with different concentrations of *A. boonei* alkaloid at 10 and 15 DAI.

This study shows that *A. boonei* leaf alkaloid has a very strong negative effect on the growth and development of *M. vitrata*. Since alkaloids, especially the indole group, are known to be strong antifeedants and repellents (Koul, 2005; Rout and Sardar, 2011; Arivoli and Tennyson, 2013), it is most likely that the significantly lower survival of the larvae in the treatments with the alkaloid was due to the antifeedant or repellent property of the compound.

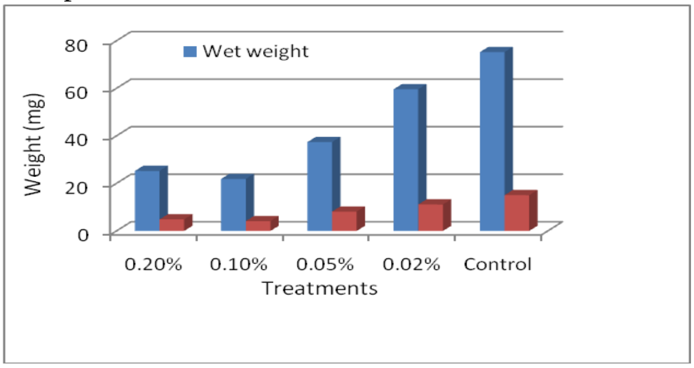

Figure 2. Mean wet and dry weights of *M. vitrata* larvae reared on artificial diet mixed with different concentrations of *A. boonei* leaf alkaloid at 10 DAI.

This is in agreement with the observation of Oparaeke (2005), who reported that the ability of *Gmelina* extracts to control *M. vitrata* and *Clavigralla tomentosicolis* Stal. might be due to its high alkaloid and tannin contents. Arivoli and Tennyson (2013) opined that the quantification of antifeedant effect of botanicals is of great importance in the field of insect pests management.
The percentage pupation at 15 DAI and adult emergence of the larval are shown in Table 1. While all the surviving larvae in the control treatment had pupated at 15 DAI, the percentage pupation ranged from 4.34 - 35.00 % in the treatments containing the alkaloid. There was no significant difference between the percentage survival in the treatments with the alkaloid. The percentage of deformed pupae however, varied between the treatments with the alkaloid. While 83.33 % of the larvae emerged as adults in the control, only a maximum of 10.00 % emerged in the treatments containing the alkaloid.

The results of this study indicate the multiple effects of *A. boonei* leaf alkaloid on the larval survival, weight, pupation and adult emergence. The pupal deformity or abnormality observed in this study may be due to some sub-lethal effects of the alkaloid. This agrees with the observation of Ntonifor *et al*. (2006), who reported that the complex mixtures of lethal and sub-lethal phytochemicals in botanicals often offer multi-factorial selective pressures on insect pests. This is why an effective biopesticide suitable for crop protection can only be developed after the mode of action of the active compound(s) is fully understood (Ba *et al*. 2009).

The very low percentage of adult emergence demonstrates the great potential of *A. boonei* leaf alkaloid in the management of *M. vitrata* on cowpea. In a related study (Oigiangbe Osawe Nathaniel, unpublished), it was observed that even the adults from the alkaloid treated diets could not lay viable eggs. According to Ntonifor (2011), botanical pesticides are of interest to organic farmers because the chemicals are natural products, hence are easily biodegradable and judged safe to handle and use on food products.

About 500 g of fresh *A. boonei* leaves is required to obtain the alkaloid needed for a single spray of 0.05 ha of cowpea field. We conclude that *A. boonei* leaf alkaloid can contribute to the search for ecofriendly biopesticides suitable for the management of *M. vitrata* on cowpea. If this happens, it will bring a great relief to the resource of poor farmers in Africa, who are finding it difficult to produce optimal yields of cowpea from their farms every year as a result of damage by this insect pest. It is hoped that further studies on *A. boonei* leaf alkaloid will lead to the development of a commercial biopesticide for the management of *M. vitrata* on cowpea in particular, and some other major insect pests in general.

Table 1. Mean* percentage pupation and adult emergence of *M. vitrata* larvae reared on artificial diet mixed with different concentrations of *A. boonei* leaf alkaloid.

Treatments	**Pupation (%)**			**Adult Emergence (%)**
	Normal	Abnormal	Total	
0.20 %	0.00[b]	12.50[a]	12.50[b]	0.00[b]
0.10 %	4.34[b]	0.00[b]	4.34[b]	4.34[b]
0.05 %	14.28[b]	14.29[a]	28.57[b]	4.76[b]
0.02 %	15.00[b]	20.00[a]	35.00[b]	10.00[b]
0.0 % (Control)	91.67[a]	0.00[b]	91.67[a]	83.33[a]

*Means followed by the same letter in columns are not significantly different from each other ($P < 0.05$; LSD).

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