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Screening of Chemical Insecticides for the Development of Pyrrolizidine Alkaloid-based Attracticides for the Management of *Zonocerus variegatus*

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Abstract: Current research has shown that derivatives of pyrrolizidine alkaloids (PA) from the dry chopped roots of *Chromolaena odorata* (Asteraceae) are effective lures for *Z. variegatus*. Bioassays on the effect of a developed PA-based attracticides on 4th instar nymphs of *Z. variegatus* were conducted in the laboratory with a completely randomized design. The PA-source was the dry roots of *C. odorata*. The PA-attracticides evaluated comprised 300 g each of dry roots of *C. odorata* poisoned with three dilutions each of three standard insecticides, Carbofuran® 3G, Perfekthion® 40 EC and Karate® 2.5 EC, each with control treatment without attracticide. The insects were exposed to cassava (serving as feed) and the PA-attracticides in choice tests. The effects of the Carbofuran® 3G based PA-attracticides in knocking down *Z. variegatus* was concentration dependent while those of Perfekthion® 40 EC and Karate® 2.5 EC were not. The results showed that treating the dry chopped roots of *C. odorata* with as low as 0.5% w/v 3G Carbofuran derives an effective PA-based attracticide for the management of *Z. variegatus*. The results indicate that poisoning the dry roots of *C. odorata* could serve as a new management strategy for *Z. variegatus*. Here, the insect will be lured to its doom rather than spraying large hectares of infested fields with pesticides. On the other hand, the use of the dry roots of *C. odorata* as a lure will be contributing to the management of the weed which impacts negatively on agriculture and forestry.

Key words: Carbofuran, *Chromolaena odorata*, insect, karate, perfekthion, pest control, weed

INTRODUCTION

Hitherto, the pestilence of the polyphagous grasshopper, *Z. variegatus* (L.) (Orthoptera: Pyrgomorphidae) was fought by synthetic insecticides (Steedman, 1990). More recently the use of myco-insecticides (Lomer *et al.*, 1999) has revolutionized the management of *Z. variegatus* in parts of Africa and to a limited extent, in Ghana. However, their adoption has been low.

In view of the economic damage caused by *Z. variegatus* and the problems associated with the control of its outbreak by conventional methods, it has become necessary of great interest to research into alternative methods of control. A simple strategy could be exploiting the sensory attraction of *Z. variegatus* to pyrrolizidine alkaloids (PAs) which they sequester as chemical defense against their predators. Since PAs are feeding stimulants for *Z. variegatus* an attracticide could be developed, for the management of nymphs of *Z. variegatus* which cause the most damage to crops in the dry season. Such an attracticide could be prepared by incorporating a low dose killing agent (chemical or myco-insecticide) into the attractant.

The manipulation of insect behavior with lures to achieve economically acceptable control of insect pests has been recognized for many years (Rothschild, 1975; Carde, 1976; Carde *et al.*, 1977; Rice and Kirsch, 1990) and more recently remarkable progress has been made with management of lepidopteran insects (Meagher, 2002; Evenden and McLaughlin, 2004). This proposed management option for *Z. variegatus* has the advantage of bringing the insect to the killing agent, thereby reducing the quantity of pesticide applied and reducing the risks associated with the direct application of chemical insecticides in particular.

The study was therefore, undertaken with the aim to develop an attracticide combining synthetic insecticide(s) with the dry roots of *C. odorata* acting as a PA-source for the effective management of *Z. variegatus*.

MATERIALS AND METHODS

Rearing of *Z. variegatus* in the Laboratory

First instar nymphs of *Z. variegatus* were collected in a fallow field at Kwadaso in the Ashanti region of Ghana and reared on cassava foliage in an insectary cage 2×2×3 m according to the method described by Cobbinah and Tuani (1992) for bioassays. The insects had no access to PAs before they were used for the bioassay.

Bioassays

Three standard insecticides, namely, Carbofuran® 3G at 1, 5 and 10 g L⁻¹, Perfekthion® 40 EC at 0.8, 2.4 and 4.0 g L⁻¹ and Karate® 2.5 EC (lambda cyhalothrin) at 0.25, 0.75 and 1.25 g L⁻¹ were used for the bioassays. The middle concentration of each insecticide was the standard recommended dose.

Each attracticide was prepared by soaking 300 g of dried chopped roots of *C. odorata* (serving as the attractant component of the attracticide) in the test insecticide solution in a 5 L capacity plastic bucket for 30 min and subsequently drying it in the sun for 3 h. The roots were dried in the sun for a minimum of 6 h for about 3 day to reduce the moisture content to about 10% before being used. The bioassays were conducted in wooden cages each measuring 0.45×0.45×0.75 m with three sides covered with grey baft material. The front of the cage served as the door. Both the door and the top of the cage were made of glass.

Potted plants of cassava, 30 cm high were provided in each cage together with each attracticide, (spread at the base of the potted cassava plant in the cage) in a choice test between feed and attracticide and replicated three times in a completely randomized block design. Three hundred grams of untreated dry chopped roots of *C. odorata* served as control in each experiment. Fifty hoppers, comprising equal numbers of both sexes of 4th instar nymphs, were introduced into each cage.

Parameters Measured and Data Analysis

Data on 'knocked down' over 96 h at 24 h intervals was measured, transformed to logits and subjected to linear regression analysis to determine the median knockdown time (KT₅₀) and median lethal dose (EC₅₀) at 48 h with GraphPad Prism® version 4.00 for windows (GraphPad Prism® 4.00, 2003). Abbot's correction formula was used in the statistical computations to account for mortalities due to other factors other than the treatments (Abbott, 1925).

Abbot's correction formula:

$$\text{Corrected\%} = \frac{N \text{ in T after treatment}}{(1 - N \text{ in Co after treatment})} \times 100$$

Where: N = number of insects, T = treated and Co = control.

RESULTS

Percentage Knock Down

The mean percentage knock down of *Z. variegatus* recorded from the various treatment concentrations of Carbofuran® 3G, Perfekthion® 40 EC and Karate® 2.5 EC are presented in Fig. 1. The percentage knock down of *Z. variegatus* after 24, 48, 72 and 96 h of exposure to each concentration

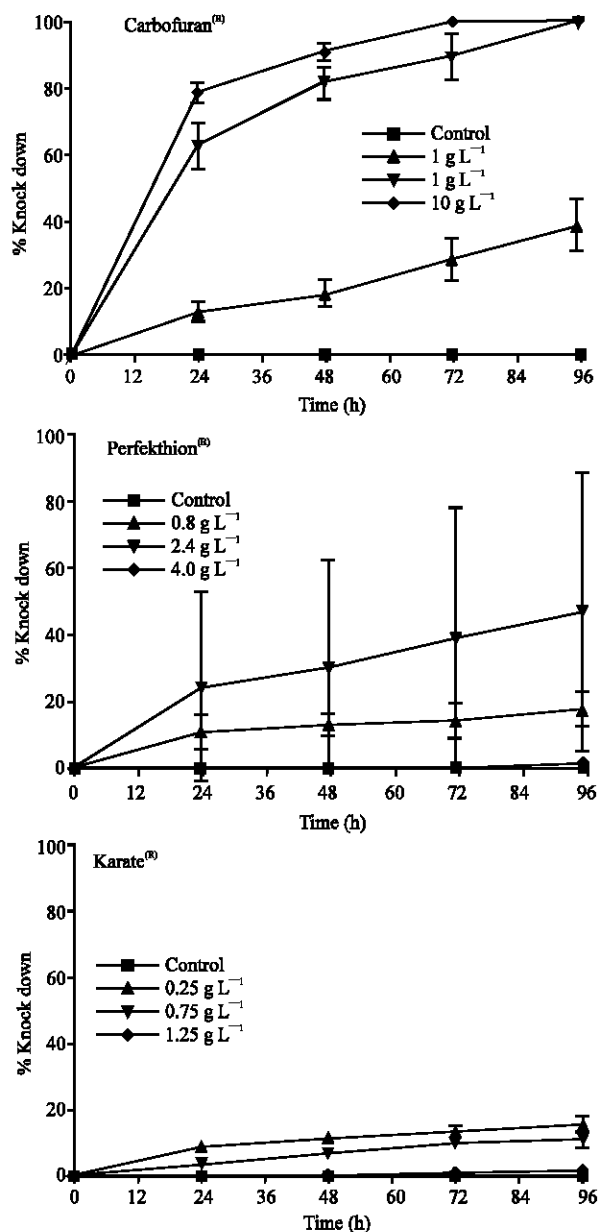


Fig. 1: Effect of doses of Carbofuran®, Perfekthion® and Karate® to pyrrolizidine alkaloid based attracticide on the mortality of 4th instar larvae of *Z. variegatus* in the laboratory. Bars indicate standard error of means

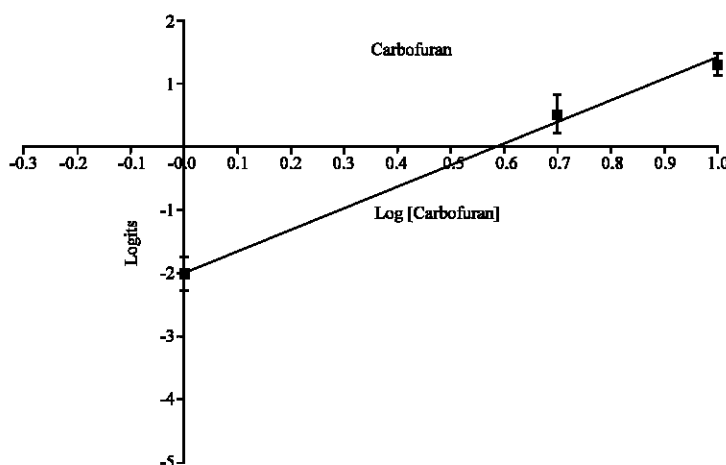


Fig. 2: Responses of 4th instar larvae of *Z. variegatus* to Carbofuran-pyrrolizidine alkaloid attracticide in the laboratory. Bars indicate standard error of means

of the PA-attracticide containing Carbofuran® 3G was significantly different from each other except for the concentrations of 5 and 10 g L⁻¹ at 96 h ($p < 0.05$). Also, the knock down progression for the Carbofuran® 3G treatment at 10 g L⁻¹ was significantly better ($p < 0.05$) than at 5 g L⁻¹, which was also significantly more efficacious in knocking down *Z. variegatus* than the 1 g L⁻¹ Carbofuran® 3G attracticide treatment. Knock down was concentration dependent.

There was an increase in knock down progression of the Perfekthion® 40 EC based attracticide from 0.8 to 2.4 g L⁻¹, but this decreased at the higher concentration of 4.0 g L⁻¹ ($p < 0.05$) (Fig. 1). With respect to the Karate® 2.5 EC based attracticide, knock down decreased beyond the concentration of 0.25 g L⁻¹ (Fig. 1).

Considering the three insecticides used, the highest knock down of *Z. variegatus* recorded was 100, 46.6 and 22.2% for Carbofuran® 3G, Perfekthion® 40 EC and Karate® 2.5 EC, respectively. The concentrations that recorded the highest mortality were 0.5 or 1% (w/v), 0.24 (w/v) and 0.25% (w/v), respectively (Fig. 1).

Median Knock Down Time

The median knock down time (KT₅₀) for *Z. variegatus* at concentrations of 0.1% (1 g L⁻¹), 0.5 (5 g L⁻¹) and 1% (10 g L⁻¹) (w/v) Carbofuran® 3G was 63, 15 and 13 h, respectively, after ingestion of the attracticide (Fig. 1), with the latter two concentrations effecting 100% knockdown at 96 h. The Perfekthion® 40 EC treatment of 2.4 g L⁻¹ recorded a KT₅₀ at 48 h.

Mean Lethal Dose

The results indicated a positive correlation ($r^2 = 0.934$) between dose and knock down with respect to the Carbofuran® 3G treatments. However, the converse was true for the Perfekthion® 40 EC and Karate® 2.5 EC treatments beyond concentrations of 2.4 and 0.25 g L⁻¹ with virtually no signs of consumption of the corresponding *C. odorata* roots. The mean dose response of *Z. variegatus* to the Carbofuran® 3G treatment at 48 h indicated a LogEC₅₀ of 0.5782, giving an EC₅₀ value of 3.79 (Fig. 2).

DISCUSSION

The results suggest that Perfekthion® 40 EC and Karate® 2.5 EC are not suitable toxicants for the development of PA attracticides for *Z. variegatus* probably due to the deterrence of their strong odours.

Unfortunately, there is no previous study to compare with the observations made in this preliminary study. However, in an unrelated study, as low as 0.16% Permethrin (a natural pyrethroid) was effective in killing *Plutella xylostella* (Lep.) on cabbage when combined with a lure (Mitchell, 2002). The concentration of Perfekthion® 40 EC which gave the best knock down results for *Z. variegatus* was 0.24% i.e., 1.5 times higher than the 0.16% used for *P. xylostella*. *Z. variegatus*, however, avoided higher doses of the Perfekthion® 40 EC thus making it unsuitable for use as a lure toxicant in its management.

The performance of Perfekthion® 40 EC and Karate® 2.5 EC beyond certain concentrations suggests some repellent activity of the insecticides on *Z. variegatus*. The strong odour of Perfekthion® 40 EC and Karate® 2.5 EC either masks the effect of the attractive PA derived volatiles emitted from the lure to attract the insects or it efficiently deters the insect. Insects which respond to pheromones/lures do have specific receptors to perceive the attractive principle in the air. It is, however, not known how the odour of Perfekthion® 40 EC and Karate® 2.5 EC may affect the odour perception of *Z. variegatus* and is worth investigating.

Generally, the lethality of the Carbofuran® 3G combined with the attractant on *Z. variegatus* turned out to be more potent than for the Perfekthion® 40 EC and Karate® 2.5 EC in decreasing order. The cassava stems (acting as feed) and the roots (primary component) of the attracticide were both eaten by *Z. variegatus*, confirming earlier observations made on the diurnal activity pattern of *Z. variegatus* to both food and PAs (Timbilla, 2006).

Symptoms of the PA-attracticide poisoning of *Z. variegatus* started as early as 15 min, with an initial sign of reduced activity of the insect and wriggling of the limbs. This behavior of the insect could continue for up to about 26 h before it died.

This technology of attract-and-kill as demonstrated in this study for the management of *Z. variegatus* with natural PAs from plant material combined with a low dose toxicant is the first of its kind and needs to be pursued further for more efficient formulations of the PA-sources as well as of the toxicants.

The use of Carbofuran® 3G and other pesticides in granular and bait formulations for the control of insects is common in North America (AAFC, 1993). However, most of the work is on lepidopteran pests (Rothschild, 1975; Carde, 1976; Carde *et al.*, 1977; Butler and Las, 1983; Haynes *et al.*, 1986; Miller *et al.*, 1990; Rice and Kirsch, 1990; Charmillot *et al.*, 2000; Krupke *et al.*, 2002; Meagher, 2002; Evenden and McLaughlin, 2004).

The low adoption and practice of attracticides in Africa has been attributed to logistical problems (AAFC, 1993). In this study, the root material used (*C. odorata*) is a weed and thus an advantage. Secondly, the cost and the amount of Carbofuran® 3G needed to prepare about 100 PA-attracticides is less than \$2.2 (i.e., \$0.02 per preparation with 300 g weight of *C. odorata*). This should be affordable to the peasant farmer, compared to spraying a whole field infested with the insect with chemical insecticide or even to use fungal preparations which are much more expensive.

Perhaps there is a huge unexplored potential for application of lures combined with killing agents for the management of many insect pest situations where current control options are inadequate for both economic and environmental reasons. The advances made in this study, using the dry chopped roots of *C. odorata* acting as a dispensing system for PA-volatiles in the attraction of *Z. variegatus* and the results achieved with Carbofuran® 3G is a remarkable step towards a cost efficient management of *Z. variegatus* in Africa.

There is a positive correlation between knock down and lethal dose with respect the use of Carbofuran® 3G in the development of the PA-based attracticide developed. Carbofuran® 3G at 0.5% (w/v) holds promise as a low dose lethal chemical insecticide for the development of PA-based attracticide for the management of *Z. variegatus*. Because of the deterrent properties, Perfekthion® 40 EC and Karate® 2.5 EC are not suitable chemical insecticides for use in the development of pyrrolizidine alkaloid attracticides for *Z. variegatus*. The management of *Z. variegatus* with the dry roots of *C. odorata* as a lure will also be contributing to the management of the weed which impacts negatively on agriculture and forestry in Africa.

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