

## Full Length Research Paper

## Insecticide irritability of plant extracts against *Sitophilus zeamais*

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Several plant species were selected as potentially safer substitutes for control of maize weevil, *Sitophilus zeamais* Motsch. The objective of the study was to investigate the insecticide irritability effect (that is, avoidance after contact) of ethanol extracts (0.05 gm.L<sup>-1</sup>) of the species *Croton heliotropiifolius* (leaves and flowers), *Duguetia furfuracea*, *Magonia pubescens*, *Senna obtusifolia*, *Senna occidentalis* and *Vernonia scabra*, in comparison with a synthetic insecticide frequently used in treatment of grains, permethrin (Piredan EC (containing 384 gL<sup>-1</sup>) DuPont do Brazil S.A., Barueri, SP, Brazil). Maize weevils of a single strain were subjected to free-choice tests with maize grains sprayed with the ethanol extracts and doses of permethrin. The behavioral response of *S. zeamais* to ethanol extracts and permethrin-sprayed surfaces differed from the control. As shown in the statistical analysis, the ethanol extract of flowers of *C. heliotropiifolius* induced significantly more contact avoidance behavior than all other plants tested. The results observed with the synthetic insecticide permethrin were similar to those obtained with *C. heliotropiifolius*, at a dose lower than recommended.

**Key words:** Maize weevil, toxicity, crude extract, velame, tingui.

### INTRODUCTION

Insects have evolved a variety of physiological and behavioral responses to various toxins in natural and managed systems. These varied responses can reflect the toxin's mode of action and the extent to which it influences pest behavior. Insects may withstand insecticide applications either through the evolution of physiological mechanisms allowing them to cope with high insecticide levels on or within the body, or through behavioral mechanisms minimizing their exposure to insecticides (Hoy et al., 1998; Jallow and Hoy, 2005).

Climatic conditions in the tropics favor the cultivation of

numerous food crops but also favor the development and proliferation of storage pests and fungal diseases, which can cause considerable damage in storage and constitute an obstacle to processing (Sousa et al., 2009; Jahromi et al., 2012). Stored-product pests are particularly important because they attack the final agricultural product. The maize weevil, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) is a serious pest of stored maize, causing qualitative and quantitative losses. Plant insecticides have played an important role in the traditional methods of protection against grain pests and

disease (Mondal and Khalequzzaman, 2010; Stancic et al., 2011; Jahromi et al., 2012).

However, synthetic insecticides are the widely used method for control of storage pests. The associated detrimental effects on the environment and health, development of genetically resistant strains, erratic supply and control failures have become a major concern and thus, given impetus to the search for alternative methods of pest control to reduce the side effects of insecticides.

Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents and ovipositor attractants, and these different activities have been observed by many researchers (Venketachalam and Jebasan, 2001; Silva et al., 2009; Ishii et al., 2010; Ntonifor et al., 2011; Suthisut et al., 2011;

Asmanizar et al., 2012). Plants are considered rich sources of bioactive chemicals and may be an alternative source of insect control agents. In recent years, research on the efficacy of plant extracts used as stored-grain protectants against insects has been intensified. The major limitation to their utilization is the high application rate ( $\text{mg}\cdot\text{ml}^{-1}$ ) required to effectively disinfest grains, which makes plant extracts an impractical and costly natural resource to apply. Using reduced levels of plant extracts, or synthesized natural compounds, in combination with the well known synthetic insecticides may turn their use more attractive and effective (Isman, 2006; Rajendran and Sriranjni, 2008; Silva et al., 2009; Ajayi and Olonisakin, 2011; Udo, 2011; Derbalah et al., 2012; Silva et al., 2012a,b).

A well written review by Nerio et al. (2010) discussed the repellent activity to grain pests of plants in families such as the Asteraceae, Myrtaceae, Labiatae and Umbelliferae. Included in the review were species known for their toxicological effects. Several plant species were selected as potential substitutes for control of the maize weevil, *S. zeamais* (Tripathi and Upadhyay, 2009), although toxicological studies are still required. The objective of the present study was to investigate the insecticide irritability effect (that is, avoidance after contact) of ethanol extracts of the selected species - *Croton heliotropiifolius* Kunth (Euphorbiaceae) (leaves and flowers), *Duguetia furfuracea* (St. Hil.) Benth and Hook (Annonaceae), *Magonia pubescens* A. St. Hil. (Sapindaceae), *Senna obtusifolia* L., *Senna occidentalis* L. (Caesalpinoidea) and *Vernonia scabra* Pers. (Asteraceae) - in comparison with permethrin (Piredan EC (containing  $384 \text{ g L}^{-1}$ ) DuPont do Brazil S.A., Barueri, SP, Brazil), a frequently used synthetic contact insecticide reported to have insect irritability effect.

## MATERIALS AND METHODS

The study was conducted in the Entomology Laboratory of the Department of Natural Sciences, Professora Cinobelina Elvas Campus, Federal University of Piauí.

## Insects

We used a single population of *S. zeamais* collected in the city of Bom Jesus (PI) - Brazil. The insects were placed in glass vessels (1.5 L) and fed whole maize grains free of insecticides and pests, under controlled temperature ( $25 \pm 2^\circ\text{C}$ ), relative humidity ( $70 \pm 5\%$ ) and photoperiod (LD 12:12).

## Collection and preparation of test materials

The extracts were obtained from leaves, flowers and stems of the six (6) plant species. We chose these species based on references to their use as an insecticide or other phytosanitary properties (Table 1).

After collection, the plant materials were washed in distilled water to remove impurities. Subsequently, they were placed in labeled paper packages and submitted to forced ventilation at a constant temperature of  $50 \pm 1^\circ\text{C}$  for drying. The dried leaves were triturated at room temperature in a mill and subjected to extraction (3 $\times$ ) with hydrated ethanol 96° GL to obtain the active chemical constituents. After evaporation of ethanol in a rotary evaporator, we obtained the crude ethanolic extract. The concentration of the ethanol extracts used was  $0.05 \text{ g mL}^{-1}$ . Commercial formulations of permethrin (Piredan EC, containing  $384 \text{ g L}^{-1}$ , DuPont do Brazil S.A., Barueri, SP, Brazil), a pyrethroid insecticide available in Brazil for use in treatment of grains, were used at their recommended label rates. The ethanol extracts were diluted in ethanol and the synthetic insecticide with water and 0.5 ml of solution was applied to the grains (50 g).

## Free-choice test of preference

Pair-wise free-choice tests were carried out using white plastic trays ( $18 \times 12 \times 4 \text{ cm}$ ), with one half containing solvent-sprayed grains (50 g) and the other half containing test materials -sprayed grains (50 g). A control with ethanol solvent-sprayed grains on both sides was also used to normalize the results. The trays were covered with a fine fabric (organza) to prevent insect escape. One hundred (100) non-sexed adult insects (1 to 2 weeks old) were released in the center of the tray and insect preference was assessed after 24 h by determining the proportion of insects present on the test materials-sprayed grains. Ten replicates were used for each of the following test materials: Crude ethanolic extract of *C. heliotropiifolius* (leaves and flowers), *D. furfuraceae*, *M. pubescens*, *S. obtusifolia*, *S. occidentalis*, *V. scabra*, and the permethrin insecticide ( $0.00025$  and  $0.025 \text{ g L}^{-1}$ , dose selected due to mortality rate of less than 70%).

## Statistical analysis

The experiments were completely randomized, with 10 treatments and 10 repetitions. The assumptions of normality, homogeneity and independence within and between variables were tested using analysis of variance (ANOVA) and Tukey's test to compare the proportion of individuals (the proportion was obtained from insects observed in the control treatment with both sides untreated) that visited the treated area and the percentage of irritability among the treatments. Student t-test was used to check for statistically significant differences in insecticide irritability among treatments.

## RESULTS

Behavior avoidance of plant extract and permethrin treated surfaces was recognized through the nine

**Table 1.** Scientific name, common name, and indication of use of each plant species studied.

Scientific name	Common name	Indication of use	Part used
<i>C. heliotropiifolius</i>	Velame	Insecticide	Leaves/flower
<i>D. furfuracea</i>	Araticum seco	Cytotoxic	Leaves
<i>S. obtusifolia</i>	Fedegoso branco	-	Leaves
<i>S. occidentalis</i>	Fedegoso	Antimalarial	Leaves
<i>M. pubescens</i>	Tingui	Insecticide	Stem
<i>V. scabra</i>	Assa-peixe	Inflammatory	Leaves

**Table 2.** Proportion of adults of maize weevil, *S. zeamais*, non-discriminating against maize grains treated with plant extracts.

Treatment	Proportion of insects in the treated area (SDM) <sup>1</sup>	Student <i>t</i> -test	<i>P</i>
Control (0)	0.500 (0.113) <sup>a</sup>	1.45	0.255
Permethrin 2.5 × 10 <sup>-4</sup> (1)	0.367 (0.064) <sup>b</sup>	4.97	0.004
<i>M. pubescens</i> (2)	0.252 (0.051) <sup>c</sup>	11.90	<0.001
<i>S. occidentalis</i> (3)	0.156 (0.042) <sup>d</sup>	17.80	<0.001
<i>Vernonia scopus</i> (4)	0.122 (0.037) <sup>d</sup>	23.16	<0.001
<i>C. heliotropiifolius</i> (5)	0.114 (0.044) <sup>ed</sup>	19.48	<0.001
<i>D. furfuracea</i> (6)	0.113 (0.035) <sup>ed</sup>	26.08	<0.001
Permethrin 2.5 × 10 <sup>-2</sup> (7)	0.104 (0.021) <sup>ed</sup>	44.13	<0.001
<i>S. obtusifolia</i> (8)	0.096 (0.014) <sup>e</sup>	22.93	<0.001
Flowers - <i>C. heliotropiifolius</i> (9)	0.029 (0.014) <sup>f</sup>	71.32	<0.001

<sup>1</sup>SDM, Standard deviation of the mean. Means followed by the same letter in the next column do not differ statistically by Tukey's test ( $p < 0.05$ ).

components used in the tests of insecticide irritability (that is, avoidance after contact). The number of insects that completely avoided contact with the insecticide-treated half of the grain was subjected to paired Student *t*-test, which demonstrated significant irritability for *S. zeamais* ( $t < 71$ ,  $p < 0.004$ ). This effect was species-specific because flowers of *C. heliotropiifolius* caused significantly more irritability behavior ( $p < 0.001$ ) in adults of *S. zeamais* than the two concentrations of the synthetic insecticide (Table 2).

All treatments (plant extracts at 0.05 g mL<sup>-1</sup> and permethrin at 2.5 × 10<sup>-4</sup> and 2.5 × 10<sup>-2</sup> gL<sup>-1</sup>) caused significant insecticide irritability in *S. zeamais* compared to untreated grains. Extract from flowers of *C. heliotropiifolius* elicited the greatest avoidance response; other treatments were approximately 50 to 90% as effective (d.f. 10; *F*. 93.3;  $p < 0.0001$ ). Insects visited the treated grain, but quickly returned and remained in the untreated area. In the treatment with permethrin 2.5 × 10<sup>-2</sup> gL<sup>-1</sup>, the percentage of irritability was approximately 80% (Figure 1).

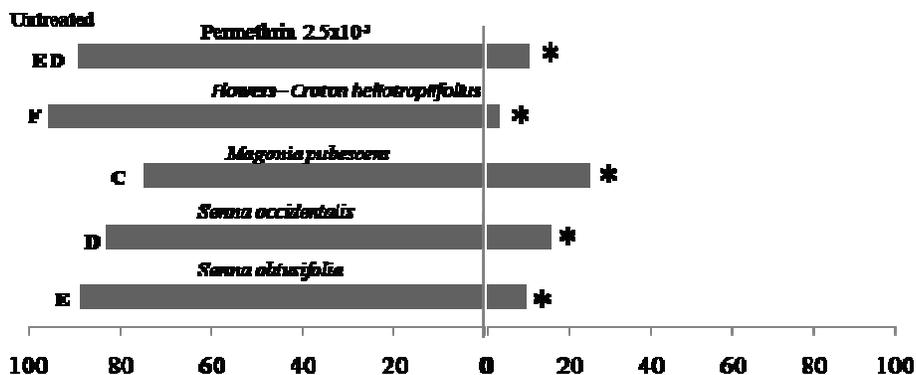
Flowers of *C. heliotropiifolius* and leaves of *S. obtusifolia* completely repelled the maize weevil within 24 h of exposure. From Figure 1, it can be concluded that permethrin, *S. obtusifolia* and flowers of *C. heliotropiifolius* had the highest irritancy effect against

adults of the maize weevil, while *M. pubescens* and *S. occidentalis* were least irritant.

## DISCUSSION

The utility of any botanical for protection against insect pests depends upon its mortality rate in the target organism and/or its sublethal effects, as on development, reproduction and behavior. The primary aim of this work was to investigate the insecticide-irritability effect of plant extract and permethrin residues on the behavior of adults of *S. zeamais*. The selection of plants was based on popular information, bactericidal activity, chemical studies and reports of insecticidal activity. Natural insecticides such as pyrethrum, nicotine and rotenone, among others, have been extensively used for insect control (Adeniyi et al., 2010). This study demonstrated that the plant extracts tested were effective against *S. zeamais* in stored maize with respect to insecticide-irritability.

Using one-way ANOVA, the results showed a significant difference among extracts of the different plant species (Table 2). Statistical analysis (Tukey's test) showed that flowers of *C. heliotropiifolius* induced the behavior to avoid the treated area to a significantly greater extent than all other materials tested against



**Figure 1.** Percentage of insects that completely avoided contact with plant extract and permethrin - treated grains. Histogram bar with asterisk indicates significant difference between treated and untreated grains by Tukey's test ( $p < 0.05$ ); histogram bars with the same letter are not significantly different by Tukey's test ( $p < 0.05$ ) between treatments.

*S. zeamais* (Table 2). All nine crude extracts tested (*C. heliotropifolius* leaves and flowers, *D. furfuraceae*, *M. pubescens*, *S. obtusifolia*, *S. occidentalis*, *V. scabra* and permethrin 0.00025 and 0.025 gL<sup>-1</sup>) induced significantly greater insecticide-irritability behavior than control.

The crude extracts of the plant species mentioned above, when impregnated in maize grains, reduced insect contact with the treated grain, as was observed after treatment with permethrin. Studies are needed to evaluate the lethal and sublethal effects of the extracts at different concentrations. In addition, the stability of the molecules responsible for the irritability and/or other insecticidal activity needs to be assessed and the mechanism(s) of action elucidated to evaluate the potential use of these substances for control of pests and/or diseases, alone or in combination.

Silva et al. (2012b) reported that the ethanol extract from leaves of *C. heliotropifolius* had contact insecticidal activity against *S. zeamais* adults. Species of this genus are known to contain insecticidal compounds such as catechin, galocatechin, diterpenes and sesquiterpenes (Alexander et al., 1991; Peres et al., 1997, 1998; Dória et al., 2010; Neves and Camara, 2012).

Knowledge of the chemical composition of plant secondary metabolites and their respective biological activities will contribute to an integrated pest management strategy. Several previous studies have demonstrated the effectiveness of different vegetable oils in protecting grains against major stored-product insect pests. It is necessary to understand the mode of action of these compounds in order to predict their usefulness for control of stored grain pests. For example, the mode of action of bioactive natural monoterpenoids (hydrocarbons, alcohols and ketones) isolated from plant extract oils may be due to inhibition of acetylcholinesterase (Miyazawa et al., 1997; Lee et al., 2001; Derbalah and Ahmed, 2011). This inhibition may be attributable largely to fumigant action (Park et al., 2003) inducing insecticide-irritability.

The crude extract of *Vernonia amygdalina* (leaf) at 4.0% concentration resulted in higher toxicity (measured as % mortality) to *Acanthoscelides obtectus* (Adeniyi et al., 2010). It was also observed that the 3.0% concentration was associated with 29.9% mortality, 2.0% with 23.9% and 1.0% with 20.3% mortality in *A. obtectus*. Adeniyi et al. (2010) also showed that extracts from leaves of *Osmium gratissimum*, *Sida acuta*, *Telfaria occidentalis* and *V. amygdalina* possess good insecticidal potential because of their phytochemical constituents. The order of toxicity of four different concentrations was 4.00 > 3.00 > 2.00 > 1.00%. Similar direct toxicity effect of the leaf extracts of plants reported here, in the order of toxicity as 5.00%, was comparable to the effect of the insecticide permethrin.

The aqueous extract of the leaves of *Vernonia* sp. has been reported to have phytotoxic properties (Alabi et al., 2005; Ijeh and Ejike, 2011). Dust from the dried leaves of *Vernonia* sp. was also found to have insecticidal potency against larvae of *Callosobruchus maculatus* (Fabricius) and *S. zeamais* - insects that cause heavy losses of stored cow pea and maize, respectively (Kabeh and Jalingo, 2007). Asawalam and Hassanali (2006) also reported that the essential oil of these plants was effective in the control of *S. zeamais*.

An insect's chance of survival may be greatly increased if its behavior is modified to avoid contact with insecticide-sprayed surfaces. The perception of insecticide presence in the environment may be achieved through insect learning ability or through genetic modifications in its peripheral receptors or central processing systems, leading to the evolution of behavioral resistance to insecticides in some species (Gould, 1991; Hoy et al., 1998).

Permethrin affects tarsal organs and its repellency effect and vapor pressure are lower than conventional repellents (Vatandosst and Borhani, 2004). On the other hand, conventional repellents affect antennal organs, and the atmosphere within the test chamber would be quickly

saturated with repellent vapor, leading to rapid habituation of the olfactory or antennal organs (Vatandosst and Borhani, 2004). Guedes et al. (2009) found that resistant strains of *S. zeamais* showed higher deltamethrin avoidance than insecticide-susceptible insects, which is evident for doses lower than the recommended label rate (0.5 ppm), where the resistant strains exhibited about 6-fold higher deltamethrin avoidance than the susceptible strain. A similar result was observed in the present study with permethrin, another active principle recommended to control pests of stored grain. Permethrin is a synthetic insecticide modeled on pyrethrins naturally occurring in flowers of some *Chrysanthemum* species. Permethrin is in the pyrethroid group of insecticides, which were designed to meet or exceed the efficacy of natural pyrethrins. At the recommended dose in the bioassay performed, permethrin caused over 50% mortality (result not shown); this result is important because it shows that lower doses than recommended can prevent grain contact by *S. zeamais* due to the irritability effect of the insecticide.

In conclusion, this study indicates that the irritability effect of permethrin, in doses lower than the recommended label rate (0.5 ppm) should be taken into consideration when used as a residual insecticide for control of *S. zeamais*, as well as impregnated in storage units. The plant extracts tested also demonstrated insecticide irritability activity, similar to that observed for permethrin. These results are promising because the extracts can be used by small farmers to avoid the damage caused by *S. zeamais* in the storage units. Changing the behavior of insect pests seems to be beneficial, because contact avoidance decreases qualitative and quantitative grain damage. Extracts of all the plants tested possess irritability activity, but it is especially pronounced with *C. heliotropiifolius* flowers; they could potentially be used to control a variety of insect pests and vectors. However, further work is necessary to elucidate the structures of the biologically active components responsible for the insecticidal activity of these plants.

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