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Toxicity of Ethanolic Extract of *Croton heliotropiifolius* in Weevil Populations of Stored Maize Grains

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ABSTRACT

The objective of this study was to evaluate, under laboratory conditions, the contact toxicity of the ethanolic extract of *Croton heliotropiifolius* on the main pest of stored maize, *Sitophilus zeamais* Motschulsky. Extract of the leaves of *C. heliotropiifolius* and maize weevil populations collected in four different storage units were used to determine whether the contact toxicity of ethanolic extract of *C. heliotropiifolius* in populations of *Sitophilus zeamais* incurs differentiated responses. After bioassays, we compared the mean mortality between the treatment and control groups. Insects were treated topically with the ethanolic extract of leaves of *C. heliotropiifolius* at a concentration of 10,000 ppm and mortality was assessed 24, 48 and 72 h after application. The mortality of *S. zeamais* adults from the four populations was significantly higher compared with to control in all evaluation periods. The mortality of *S. zeamais* adults (<15 days-old) topically treated with the extract increased with time of exposure to extract residues. The populations evaluated had different susceptibility in relation to contact with the ethanolic extract studied. According to the results obtained, it was concluded that the ethanolic extract of leaves of *C. heliotropiifolius* has contact insecticidal activity against adults of *Sitophilus zeamais* and that the response to this toxicity varies with the geographical origin of the population analyzed.

Key words: Botanical insecticide, *Sitophilus zeamais*, *Croton heliotropiifolius*, Euphorbiaceae, stored maize grain

INTRODUCTION

Post-harvest grain loss is known to be high and unrecoverable because it is the final product. In the storage phase specifically, grain losses oscillate between 1.5 and 50%, varying mainly by country. In more developed countries they oscillate at levels below 10% (around 5% in the U.S.), while in developing countries like Brazil losses may reach 50% (FAO, 1994; Worley, 2002; Adam *et al.*, 2006; Santos, 2011).

Mainly responsible for this damage are the insect pest of stored grain, especially the weevils (Santos *et al.*, 1986; Guedes, 1990; FAO, 1994; Adam *et al.*, 2006). Insects found in stored products show a wide variation in population levels. This is probably the result of management of diverse pests that, consistent with the region and discontinuous nature of the storage environment, accentuate seasonal population cycles leading to population bottlenecks where only a small number of individuals establish new populations (Tran and Credland, 1995; Guedes *et al.*, 1997, 2003, 2007, 2009). This diversity has implications for the management of these species, because there are differences in the consumption (and losses) of grain caused by them. In addition, there are differences in the efficiency of the different control methods used to manage these pests (Guedes *et al.*, 1997, 2003, 2007, 2009; Fragoso *et al.*, 2005; Pimentel *et al.*, 2007). Thus, the study of variation in response to the alternatives for control of these insects can be a source of important tools for integrated management.

The main method of control of weevils in stored grain is the use of insecticides, because it is the simplest method of control. Insecticides offer a fast and economical way to contain infestations of pests of stored products, mainly in tropical areas, due to the lack of alternative methods for control of populations of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). The frequent use of insecticides to protect stored grain from insects has resulted in the development of resistance to these compounds in these pests and control failures have been observed in several countries (Subramanyam and Hagstrum, 1995), including Brazil (Ribeiro *et al.*, 2003).

Management alternatives are necessary for insect pests of stored products, particularly considering the scope of the problems of insecticide resistance. Currently few conventional insecticides are permitted to be used against these pests. The limited number of control agents available drives the exploration of alternative compounds to conventional insecticides, which are particularly important for family farms, but could also present viable solutions for large storage units. In this context, reports of insecticidal activity of different plant species (Vieira *et al.*, 2001; Moreira *et al.*, 2005, 2007; Rosell *et al.*, 2008; Rajendran and Sriranjini, 2008) should be considered in the search for biopesticides to replace not only conventional insecticides but also fumigants.

Studies on the toxicity of plant extracts to populations of *S. zeamais* contribute to guide actions that can be used in phytochemical studies and management of insect pests. For this reason, we sought to assess in this study whether the contact toxicity of ethanolic extract of *C. heliotropiifolius* Kunth (Euphorbiaceae) in populations of *S. zeamais* incurs differentiated responses.

MATERIALS AND METHODS

Populations of *S. zeamais* were subjected to exposure by contact with ethanolic extract of *C. heliotropiifolius* to identify the insecticidal potential of this plant species.

Insects: We used four populations of *S. zeamais* collected in the cities of Bom Jesus (PI) Brazil, Redencao (PI), Volta Redonda (RJ) and São Jose do Rio Pardo (SP). The populations were multiplied and maintained in 1.5 L glass containers at a temperature of 27±2°C and relative humidity of 70±10%.

The populations were fed grains of maize stored under refrigeration to avoid infestation. The maize was removed from the freezer and allowed to equilibrate to room temperature, approximately 27°C, before feeding.

Plant extract: Leaves of *C. heliotropiifolius* were collected in the first half of May 2010 in the city of Bom Jesus in southern Piaui state, Brazil. The species was identified by Dra. Inês Cordeiro, Institute of Botany.

The dried leaves were triturated at room temperature in a mill and subjected to extraction (3x) with hydrated ethanol (96%) to obtain the active chemical constituents. After evaporation of ethanol in a rotary evaporator, there remained a green solid, designated "ethanolic extract".

Bioassay topical application: The volume of 50.0 µL of ethanolic extract of *C. heliotropiifolius*, at a concentration of 10,000 ppm, was applied to the dorsal region of *S. zeamais* using an automatic pipette. The experimental unit consisted of groups of 20 adult insects (<15 days-old) treated individually with 50 µL of extract and then confined in petri dishes. The controls received only the ethanol solvent. The experiment was repeated 10 times for each population. Mortality was assessed after 24, 48 and 72 h.

Statistical analysis: We applied analysis of variance (PROC GLM, MANOVA; SAS, 2002), followed by univariate analysis of variance and Tukey's HSD test, when appropriate (SAS, 2002) to compare the mean proportion of mortality among the populations studied in bioassay the topical toxicity. We used the Student's t-test to compare the effect of ethanolic extract versus control for each assessment time. Regression equations of mortality as a function of time of application were used to determine the lethal time for 50 and 90% of the population. All results were analyzed considering a significance level of 5%.

RESULTS

The mortality of *S. zeamais* adults from four populations topically treated with 10,000 ppm of ethanolic extract from *C. heliotropiifolius* was significantly higher compared to control in all periods of observation, by Student's t-test at 5% probability (Table 1).

The populations evaluated had different susceptibility in relation to contact with the ethanolic extract of *C. heliotropiifolius* at a concentration of 10,000 ppm (Table 1). The highest observed

Table 1: Average mortality of adults of *Sitophilus zeamais* from four populations, toxicity caused by contact with ethanolic extract of *Croton heliotropiifolius*

		Treatment				
Population	Hours after application	Control (a)	10.000 ppm (b)	t-value	p-value	Comparison
Redencao	24	1.00 ± 1.150^{a}	4.00 ± 2.58^{ab}	-3.35	0.0040	a<b
	48	1.70 ± 1.700^{a}	8.00 ± 2.91^{a}	-5.92	<0.0010	a<b
	72	2.90 ± 2.690^{a}	18.80 ± 1.62^{a}	-16.03	<0.0010	a<b
Bom Jesus	24	0.80 ± 0.920^{a}	4.70 ± 1.49^{a}	-7.03	<0.0010	a<b
	48	1.60 ± 1.580^{a}	13.20 ± 2.30^{b}	-13.15	<0.0010	a<b
	72	2.80 ± 2.970^{a}	17.20 ± 1.81^{a}	-13.07	<0.0010	a<b
Volta Redonda	24	0.50 ± 0.850^{a}	4.18 ± 1.60^{ab}	-6.48	<0.0010	a<b
	48	2.60 ± 1.900^{a}	4.64 ± 1.57^{c}	-2.69	0.0140	a<b
	72	3.20 ± 2.490^{a}	5.64 ± 1.96^{b}	-2.51	0.0220	a<b
Rio Pardo	24	0.70 ± 0.820^{a}	2.10 ± 1.52^{b}	-2.56	0.0200	a<b
	48	1.50 ± 1.270^{a}	5.00 ± 1.56^{c}	-5.50	<0.0010	a<b
	72	2.00 ± 1.760^{a}	8.20 ± 2.10^{c}	-7.15	<0.0010	a<b

Values are Mean±SD, a<b: Significant difference by Student's t-test at p<0.05. Mean mortality individuals followed by the same letter in the column indicates no difference between the populations as a function of exposure time, by Tukey's test (p>0.05), N = 200

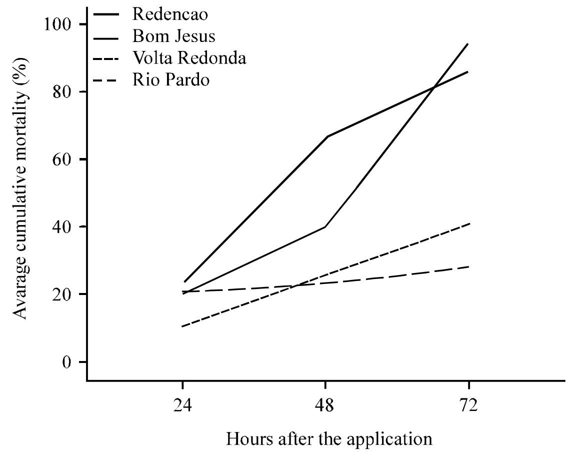

Fig. 1: Cumulative average percentage mortality as a function of exposure time to extract of *Croton heliotropiifolius* in four populations of *Sitophilus zeamais*

mortality was in populations from Bom Jesus and Redencao- more than 80% throughout the period of exposure to residues of the extract. All populations topically treated with 10,000 ppm of extract of *C. heliotropiifolius* showed a mortality rate above 20% (Fig. 1), indicating that the extract contains compounds that decrease the survival of the insects.

The mortality of *S. zeamais* adults topically treated with extract of *C. heliotropiifolius* at a concentration of 10,000 ppm increased as a function of time of exposure to chemical constituents of the extract (Fig. 1, Table 1).

Through the data presented in Table 1, it appears that in the case of controls, there was no significant difference in the average mortality of *S. zeamais* among the populations studied at any observation time after the start of the experiment. After 24 h lower mortality was observed in the population from Rio Pardo relative to Bom Jesus. After 48 h, the average mortality for the populations from Volta Redonda and Rio Pardo was significantly lower than for the Redencao and Bom Jesus and there was no significant difference between them. The average mortality of Redencao was significantly lower than that of Bom Jesus. After 72 h, the mean mortality in *S. zeamais* populations from Redencao and Bom Jesus was higher and their values were similar, while mortality in the population from Volta Redonda was lower and significantly less than that of Rio Pardo. Considering the extent of the toxicity of the *C. heliotropiifolius* extract, it can be concluded that individuals in the population from Volta Redonda are more resistant to the toxic effect of the extract at a concentration of 10,000 ppm.

The population with lethal time before TL_{50} value was Bom Jesus (41 h), while the after TL_{50} was observed in the population from Volta Redonda, with a lethal time of 441 h to kill 50% of individuals (Table 2).

The order of susceptibility of populations based on TL_{50} shows there is a difference in the response with regard to toxicity, when comparing the four populations of *Sitophilus zeamais* topically treated with the extract *C. heliotropiifolius* at a concentration of 10,000 ppm. It is important to investigate the mechanism of action of compounds from plants with insecticidal activity and correlate the mechanism of action of these compounds with commercial insecticides used in

Table 2: Regression equation for percentage of mortality, using as variable hours after application

Populations	Independent variable	Regression coefficient	SD	Significance	TL_{50} (h)	TL_{90} (h)
Redencao	Intercept	-22.600	7.02	0.003	47.0	73
	Hours	1.540	0.13	<0.001		
	R^2	0.820				
Bom Jesus	Intercept	-4.000	5.23	0.450	41.5	72
	Hours	1.300	0.10	<0.001		
	R^2	0.850				
Volta Redonda	Intercept	15.500	4.17	0.001	441.5	704
	Hours	0.170	0.08	0.048		
	R^2	0.133				
Rio Pardo	Intercept	-5.000	4.15	0.238	71.0	134
	Hours	0.635	0.08	<0.001		
	R^2	0.692				

LT: Lethal time

control of the maize weevil. Different responses of maize weevil populations have been well documented, as regards resistance to pyrethroids and organophosphates insecticides (Pereira *et al.*, 2009; Correa *et al.*, 2010).

DISCUSSION

A high percentage of mortality in *S. zeamais* adults was observed in populations from Bom Jesus and Redencao, when treated topically with the ethanolic extract of *C. heliotropiifolius*. This toxicity can be attributed to the chemical composition of leaves of *C. heliotropiifolius*.

Species of this genus are known to contain insecticidal compounds such as catechin, gallocatechin, diterpenes and sesquiterpenes (Alexander *et al.*, 1991; Peres *et al.*, 1997, 1998; Doria *et al.*, 2010; Neves and da Camara, 2012). Similarly, the ethyl acetate fraction of the species *Croton urucurana* caused 100% mortality in third instar larvae of *Anagasta kuehniella* and when incorporated into artificial diet and fed to third instar nymphs of *Dysdercus maurus* before the third day (Silva *et al.*, 2009, 2012). The major component of *C. heliotropiifolius* essential oil was identified as **β**-caryophyllene, which was effective against *A. aegypti* (LC_{50} 544 ppm). The authors suggested that the larvicidal potential is due to the existence of a synergistic effect of minor components in the essential oils (Doria *et al.*, 2010). Croton species have high insecticidal power and in some cases are more toxic to insects than pyrethrum, a substance already commercialized in several parts of the world as an insecticide and found in chrysanthemum flowers (Guerra, 1985). Moreover, Couto and Sigrist (1995) commented that pyrethrum has a broad spectrum of insecticidal action, an observation consistent with the results obtained by Alexander *et al.* (1991), Kubo *et al.* (1991) and Morais *et al.* (2006).

Ntonifor *et al.* (2011) and Abdullahi *et al.* (2011) reported a high percentage of mortality of *Sitophilus zeamais* after treatment with plant extracts of *Chenopodium ambrosioides* and oil of lemon peel, respectively, at different concentrations. They observed that mortality increases with time of exposure to compounds present in the extracts, consistent with the results reported in this study, in which the highest percentage of mortality was observed 72 h after exposure via topical application in the four populations studied.

Research on responses of populations of insect pests of stored grains has been documented frequently in the last two decades (Subramanyam and Hagstrum, 1995; Guedes *et al.*, 1997, 2003, 2007, 2009; Guedes and Zhu, 1998; Lorini and Galley, 1999; Perez-Mendoza, 1999;

Ribeiro *et al*., 2003). In the present study, the results obtained in topical application bioassays show there is a difference in the response of populations to the same compound. Comparing the four populations, two were collected in the South of Piaui, where use of insecticides for pests of stored grain is low and the other two were collected in the Southeast and Midwest, where there is constant use of insecticides to control pests of stored grains. The latter populations were less susceptible to the effect of *C. heliotropiifolius* and variation between the two was low. The difference in the response of populations from Rio Pardo and Volta Redonda relative to Bom Jesus and Redencao may result from the genetic origin of resistance in these populations to pyrethroid insecticides and phosphorous (Pereira *et al*., 2009; Correa *et al*., 2010).

Correa *et al*. (2010) observed that there is a difference in the response of male and female insects from the same population. The authors observed that the proportion of time spent in the untreated half is significantly higher in females compared to males. They also observed variations in responses between the populations. Females obtained from the cities of Vicosa and Canarana presented a lower proportion of time spent in relation to males.

Mortality bioassays to verify the toxicity of plant extracts are important in guiding future research for the development of new and effective products to control pests of stored grains. Data on the response of different populations are important to guide the development of new products as well as assist in integrated pest management.

The large differences observed among the maize weevil populations result from management of these insect pests and the discontinuous nature of the grain storage process. Storage units probably accentuate the seasonal cycles of these populations, enabling the rapid establishment of new populations from a small number of individuals (Tran and Credland, 1995). Some researchers report that this interpopulational variation could be caused by differences in sensory processes (Desneux *et al*., 2007; Hoy *et al*., 1998).

According to the results presented here, it is concluded that the ethanolic extract of leaves of *C. heliotropiifolius* has contact insecticidal activity against *Sitophilus zeamais* adults and the response to this toxicity varies with the geographic origin of the population analyzed. At a concentration of 10,000 ppm, all populations topically treated with the extract of *C. heliotropiifolius* had a higher mortality rate than the control.

The ethanolic extract of leaves of *C. urucurana* has insecticidal activity against *Anagasta kuehniella* and *Dysdercus maurus* (Silva *et al*., 2009, 2012). Species of this genus are known to contain insecticidal compounds such as catechin, gallocatechin, diterpenes and sesquiterpenes (Alexander *et al*., 1991; Peres *et al*., 1997, 1998; Neves and da Camara, 2012).

The ethanolic extract of leaves of *C. heliotropiifolius* can be incorporated into alternative methods of pest control of stored grains, assisting small farmers, so that they can develop their own methods of control based on cost-benefit, sustainability and ecological balance and thus develop alternative formulations of pesticides to control or supplement conventional insecticides. Obviously, efficacy and safety testing for new post-harvest product formulations is required before large-scale use. However, since *C. heliotropiifolius* is often used as a medicinal plant, it is unlikely to cause damage to the environment or safety issues.

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