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Insecticidal activity of *Vitex cymosa* (Lamiaceae) and *Eschweilera pedicellata* (Lecythidaceae) extracts against *Sitophilus zeamais* adults (Curculionidae)

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Abstract

This study evaluated insecticidal and repellent effects of *Vitex cymosa* and *Eschweilera pedicellata* extracts against *Sitophilus zeamais* adults. Contact on filter paper discs and contaminated grain ingestion assays were performed. The repellent effect was evaluated with the "preferential area" method. The extracts provided good results by ingestion and as repellents, but not by contact. *V. cymosa* branches methanol extract was the best, killing nearly 70% of the individuals at its highest concentration, followed by *V. cymosa* flowers dichloromethane extract and *E. pedicellata* branches aqueous extract. Among these, only *V. cymosa* leaves dichloromethane extract did not reduce the number of individuals in F1. Analyzing the repellent effect, when the variable concentration was taken into account, no extract was dose-dependent, and the intensity of response varied with the time interval. Among the extracts tested, *V. cymosa* branches methanol extract is the most promising one, which negative effect on parental resulted in F1 decrease number and the ingestion way was the most efficient.

Key words: Botanical insecticides, Plant extracts, Stored grain pests, Weevil

Introduction

Sitophilus zeamais Motschulsky, 1855 (Coleoptera, Curculionidae) is considered an important stored grains pest in Brazil. This weevil affects production quantitatively and qualitatively, and contaminates grain with excrement and exuviae (Gallo et al., 2002). Even today, its control is based on the successive application of synthetic insecticides (Lazzari and Lazzari, 2009). However, the continued use of these products has created serious problems for the environment and human health (Lara, 1991). In order to reduce such problems, alternative control measures have been adopted (Viegas Júnior, 2003). Substances with insecticidal properties, metabolized and released by plants and capable to act upon insect targets, are an important resource against insect pests (Lara, 1991; Viegas Júnior, 2003). These substances, known as secondary metabolites, have been studied for their insecticidal potential, mainly because of the advantages to the environment and the organisms, and above all because of their proven efficiency against pests (Viegas Júnior, 2003; Zarbin et al., 2009).

Several studies have confirmed plant extracts insecticidal activity against *S. zeamais* adults (Asawalam et al., 2006; Arannilewa and Odeymi, 2007; Liu et al., 2007; Llanos et al., 2008; Akob and Ewete, 2009).

Studies on the insecticidal activity of the two plant species essayed in the present work were not found in the literature available. However, some studies on other species of the genus were conducted by Hebbalkar et al. (1992), Mehlhorn et al. (2005), Rodríguez-López et al. (2007) and

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Karunamoorth et al. (2008). The possibility of finding chemical and bioactive properties in the genus moved us to evaluate the insecticidal activity of *Vitex cymosa* Bertero ex Spreng. (Lamiaceae) and *Eschweilera pedicellata* (Rich.) SA Mori (Lecythidaceae) plant extracts against *S. zeamais* adults. Considering the good results of botanical insecticides in the control of *S. zeamais* there is a good perspective of reconciling agricultural productivity with ecological considerations.

Materials and Methods

Plant material

V. cymosa was collected on Marchantaria Island, Catalão Lake, Amazonas, Brazil (3º10'04''S/59º54'45''W) in July/2004 and *E. pedicellata* was collected on Reserva Ducke, Amazonas, Brazil (2°58'6.25''S/59°55'49.96 ''W) in April/2005. Their identification was made at INPA's Botanical Research Coordination Herbarium and a voucher specimen of each was deposited, under the numbers: 230695 and 230696, respectively. Each vegetal part was dryed at room temperature, ground and extracted first with dichloromethane, followed by methanol, then hydromethanol (methanol/water 7:3) and finally with distillated water. Each extraction was performed three times, by using ultra-sound for 20 minutes. After filtration, the organic extracts were concentrated on rota-evaporator and the aqueous were lyophilized. All were kept on freezer -20°C, until essayed.

Assays

Bioassays were performed at the Agricultural Entomology Laboratory of the National Institute of Amazon Research - INPA, Amazonas, Brazil, at a temperature of 25±2°C, 60±10% relative humidity, and a 12:12 light:dark photoperiod, as proposed by Tavares and Vendramim (2005). All *S. zeamais* adults used in the experiments were obtained from a stock insect rearing from the same laboratory.

Repellence assay

The repellence assay was based on the "preferential area" method, adapted from McDonald et al. (1970). Sheets of filter paper (diameter 9.0 cm) were cut in halves, and three aliquots of 0.5 mL of each of the extracts, at the concentrations of 10, 30 and 50 mg/mL were applied to one of the halves (Table 1), the other half receiving no treatment. In control plates, 0.5 mL of specific solvent was applied to one of the halves: DCM for dichloromethane extracts, MeOH for methanol and hydroalcoholic extracts, and distilled water for aqueous extracts, the other half receiving no treatment. The treated and control half-discs were air dried until complete solvent evaporation. Then, the filter paper halves were placed juxtaposed in Petri dishes and joined with adhesive tape. In the center of each plate, twenty unsexed adults of ages between 10 and 20 days were released, and the number of individuals in each half was recorded after 30 minutes, 1, 2, 24 and 48 hours. Each treatment and control was performed in ten replicates.

Contact assay with impregnated filter paper

The methodology used for contact assay with impregnated filter paper was proposed by Huang et al. (1997), with modifications. Sheets of filter paper (diameter 9.0 cm) were impregnated with 1 mL of each extract's concentration as shown in Table 2 and placed into Petri dishes, while control sheets were impregnated with 1 mL of the specific solvent, as indicated for the repellence assay. The filter paper discs impregnated with extracts and solvents were air dried until complete solvent evaporation. Then, they were placed into Petri dishes (diameter 9.0 cm), and twenty unsexed adults of ages between 10 and 20 days were put in each treatment and control dish, totalizing five replicates per treatment and control plate. The plates were wrapped in plastic film to prevent escape. Five days after exposure, dead insects were counted, death being ascertained by the complete absence of movement.

Contaminated grain ingestion assay

The contaminated grain ingestion assay was performed as described by Llanos et al. (2008), with modifications. Twenty grams of commercial maize, hard group, type 2, were weighed and put into 500 mL vials (diameter 9.0 cm, depth 2.5 cm). Treatment vials were impregnated with 2 mL of extract in the three concentrations (Table 2), in the control group, 2 mL of the specific solvent were applied, as for the repellence assay. The grain mass was manually mixed and air dried until complete solvent evaporation. Twenty unsexed adults with ages comprised between 10 and 20 days were put into each control and treatment vial. The dead individual's number was counted fifteen days after the beginning of the experiment, total absence of movement was adopted as the mortality criteria.

Effect of extracts on adult emergence in the F1 generation

Once the contaminated grain ingestion assay was finished, all adults, dead and alive, were removed from treated and control vials, and the grain was stored for 49 additional days (7 weeks) at the laboratory for evaluation of reduction

percentage of the number of individuals emerged in the F1generation (Arannilewa and Odeyemi, 2007). At the end of this period, the emerged individuals in vials treated with extract concentrations and in control vials were counted and compared. The value obtained was used as the number of emerged individuals in the F1 generation reduction percentage, calculated with the formula proposed by Arannilewa and Odeyemi (2007):

% Reduction = 100 - (Et / Ec) x 100

where: Et = number of emerged adults in treated samples

Ec = number of emerged adults in control group.

Data analysis

Repellency Percent (RP) was calculated as proposed by Asawalam et al. (2006):

RP = [(Nc + Nt) x (Nc - Nt)] / 100

where: Nc = number of insects in control group

Nt = number of insects in treatment

RP values were evaluated against extract concentration and exposition time using one-way ANOVA. The averages obtained for each concentration where significant probabilities (<0.05) were observed were subjected to Dunnett's test (α = 0.05) in order to assess if any concentration had a greater effect. Analyses were run in JMP 4.2.0 SAS Institute, Cary, NC, USA. The effect of extract concentration (by contact and ingestion) on *S. zeamais* adult survival was analyzed with the one-way Kruskal-Wallis test, with a post-treatment with the Dunn test to obtain multiple comparisons between experimental groups. Statistical methods were applied using Microsoft® Excel 2007 electronic spreadsheets (Zar, 1999). Finally, median lethal concentrations (LC_{50}) and confidence intervals were estimated by the Trimmed Spearman-Karber method with the SAISA (2008) program.

Results and Discussion

Plants secondary metabolism substances may elicit avoidance/attraction on insect pests, such responses are triggered by olfactory perception of volatile substances, which may trigger a response of avoidance (if the substance is a repellent) or attraction (if the substance somehow attracts the insect) (Chapman, 1969). Such substances are abundant in essential oils, and have been reported for modify insect behavior (Tapondjou et al., 2005).

However, the relationship of volatile substances with the avoidance/attractive effect does not invalidate that pest insects may respond to contact with plant extracts. What happen is that while oils have been extensively tested, considered the repellent effect, extracts has rarely been assessed (Viglianco et al., 2008; Akob and Ewete, 2009). In the present work, *E. pedicellata* leaves aqueous extract showed repellent activity after 2 hours at the 1 mg/mL concentration. *E. pedicellata* branches aqueous extract at 30 mg/mL concentration and 2 hours was repellent too and that extract showed activity after 24 hours at 50 mg/mL concentration (Table 1).

To *V. cymosa* extracts, flowers methanol extract was repellent after 1 and 2 hours, and in both cases, the effect was achieved at the highest concentration. Likewise, leaves hydroalcoholic extract repelled adults at the maximum concentration, however, this response was faster, after 30 minutes. In its turn, the lowest concentrations of roots hydroalcoholic extract repelled adults after 2 hours. So, considering all time intervals, the response was not the same, similar result was showed by Viglianco et al. (2008). And, considering each concentration, none of the extracts tested showed dose-dependent effect (Table 1), however, a dose-dependent response of individuals has been observed (Babarinde et al., 2008; Akob and Ewete, 2009).

Chapman (1969), propose that the insects' repellent/attractive response depends on the chemical composition of extracts in association with the individuals' olfactory perception. If individuals' responses depend on olfactory perception, then the change in environmental circumstances, which occur naturally, mainly changes in temperature, that might have influenced the not common results showed in this work (Tables 1 and 2). Besides that, the individuals used in the assay were from a young creation, in this case, very heterogeneous (Haddad, 1998), which may also have influenced.

Still on olfactory perception, repellent effect was not seen after 48 hours (Table 1), this lack of response could be due to the loss of volatile molecules in the extracts. Thorhill and Alcock (1983) classified the durability of olfactory stimuli perceived by insects in search for hosts from short to long. It is thus possible that the substance(s) detected by individuals in the present study are not persistent in the environment. As the extracts tested tend to lose their effectiveness in 48 hours, we suggest that they be tested in future assays over longer periods at a lower temperature, or maybe with microencapsulated formulations, which slow down the release of active ingredients.

Table 1. Repellent effect of *Vitex cymosa* and *Eschweilera pedicellata* extracts against *Sitophilus zeamais* adults. Data submitted to *Dunnett's*.
*F=statistical value; DF=degrees of freedom; P=probability **NS = not significant.

Plant	Part	Extract	Concentration (mg/mL)	30 min			1 h			2 h			24 h			48 h		
				*F	DF	P	F	DF	P	F	DF	P	F	DF	P	F	DF	P
E. pedicellata	Leaves	Aqueous	1	**NS	NS	NS	NS	NS	NS	4.4549	3	0.0092	NS	NS	NS	NS	NS	NS
			30	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			50	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
E. pedicellata	Branches	Aqueous	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			30	NS	NS	NS	NS	NS	NS	3.2018	3	0.0347	NS	NS	NS	NS	NS	NS
			50	NS	NS	NS	NS	NS	NS	NS	NS	NS	2.8835	3	0.0491	NS	NS	NS
V. cymosa	Flowers	Methanolic	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			30	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			50	NS	NS	NS	3.3102	3	0.0308	5.2462	3	0.0042	NS	NS	NS	NS	NS	NS
V. cymosa	Leaves	Dichloromethanic	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			30	NS	NS	NS	NS	NS	NS	3.8329	3	0.0176	NS	NS	NS	NS	NS	NS
			50	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
V. cymosa	Leaves	Aqueous	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			30	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			50	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
V. cymosa	Leaves	Hydroalcoholic	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			30	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			50	3.9487	3	0.0156	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
V. cymosa	Leaves	Methanol	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			30	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			50	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
V. cymosa	Branches	Methanol	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			30	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			50	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
V. cymosa	Roots	Hydroalcoholic	1	NS	NS	NS	NS	NS	NS	3.404	3	0.0279	NS	NS	NS	NS	NS	NS
			30	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			50	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
V. cymosa	Roots	Methanol	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			30	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
			50	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 2. Percentage of *Sitophilus zeamais* adult's dead by contact and ingestion assays and effect of extracts on adult emergence in the F1 generation.

Species		*Eschweilera pedicellata*												*Vitex cymosa*															
Plant Part		Leaves						Branches						Branches						Leaves						Flowers			
Extract		Aqueous						Aqueous						Aqueous						Aqueous						Aqueous			
Concentration (mg/mL)		1	5	10	20	30	50	1	5	10	20	30	50	0,7	3,3	6,7	16,7	23,30	43,30	1	5	10	20	30	50	0,3	0,5	6,7	13,3	26,7	50
Dead adults (%)	Contact	2	2	0	0	0	2	0	1	1	5	2	2	9	3	4	3	3	0	0	3	1	1	0	3	1	2	2	4	7	0
	Ingestion	0	0	0	0	0	0	0	0	0	0	19	13	0	0	0	0	0	0	19	4	7	2	0	0	1	0	0	0	0	0
Individuals reduced in F1 (%)		-55	55	55	42	-48	16	-22	-64	-12	36	73	68	-62	8,7	-53	53	24	21	35	1,7	10	35	15	5,8	17	23	20	19	19	10

Species		*Vitex cymosa*																											
Plant Part		Stems						Rachis						Leaves						Roots						Flowers			
Extract		Aqueous						Aqueous						Hydroalcoholic						Hydroalcoholic						Methanol			
Concentration (mg/mL)		1	5	10	20	30	50	1	5	10	20	30	50	1	5	10	20	30	50	1	5	10	20	30	50	1	5	10	20	30	50
Dead adults (%)	Contact	2	1	1	1	1	2	2	2	1	3	4	2	0	2	0	0	2	0	0	3	2	0	4	0	0	0	4	1	2	2
	Ingestion	0	0	0	0	0	0	0	0	0	0	0	0	0	1	8	5	2	7	7	0	7	0	3	7	1	0	2	0	5	1
Individuals reduced in F1 (%)		-8	34	59	40	58	62	-62	8,7	53	53	24	21	6,8	-4	16	-3	3,2	4,8	2,4	8	-6	-1,2	-13	16	-5	-6	-5	-10	-10	1,2

Species		*Vitex cymosa*																							
Plant Part		Branches						Leaves						Leaves						Flowers					
Extract		Methanol						Methanol						Dichloromethane						Dichloromethane					
Concentration (mg/mL)		1	5	10	20	30	50	1	5	10	20	30	50	1	5	10	20	30	50	1	5	10	20	30	50
Dead adults (%)	Contact	0	2	0	4	2	5	0	0	0	0	0	0	2	0	2	4	5	4	2	2	2	2	1	0
	Ingestion	36	56	43	46	72	69	2	0	4	0	2	0	10	2	13	4	6	10	25	31	22	12	17	18
Individuals reduced in F1 (%)		28	34	15	8,4	70	35	-13	3,6	-0	1,6	-3	-1	13	5,1	31	9,7	35	-5	15	21	25	27	26	28

Nevertheless, by contact, the extracts tested did not show a toxic effect, since the most active extract did not kill more than 10% of the individuals (Table 2). Notwithstanding, when evaluating insecticidal activity, the fraction of dead individuals can be low (Silva-Aguayao et al., 2005; Liu et al., 2007), especially because the results depend on the exposure method, extraction type, defense mechanisms developed by the target organisms, and concentration and chemical extract composition, the latter by its turn being dependent on physical and chemical environmental factors (Isman, 2000).

On the other hand, better results via ingestion were recorded, mainly with *V. cymosa* branches methanol extract, which killed 36% of individuals even at its lowest concentration, this value ascending to about 70% when the concentration was highest, followed by *V. cymosa* flowers and leaves dichloromethane extracts and *E. pedicellata* branches aqueous extract (Table 2). The best results by ingestion may be directly related to the chemical composition of the integument.

The integument confers chemical, biological and mechanical protection, functioning as a protective barrier to excessive water loss and the entrance of parasites and insecticidal substances (Chapman, 1969; Gallo et al., 2002). Thus, the low effect by contact assay could be related to the impermeability of the integument to insecticidal molecules present in the extracts. Since the substances present in extracts can either remain in the grain outer layers or penetrate them, and that the Curculionidae individuals pierce plant structures, the best results obtained by ingestion could mean that the insecticide molecules penetrated the grains. The comparative analysis of the ingestion results confirmed the greater effect of *V. cymosa* branches methanol extract (Table 3).

Table 3. Statistical analysis of *Sitophilus zeamais* adult mortality by ingestion assay, made by *Kruskal-Wallis* method, with post-treatment by the method of *Dunn*, considering each plant part, extract and solvent.

Group	Identification	R_i	$\overline{R}$	Groups													
				14	13	12	11	10	09	08	07	06	05	04	03	02	01
14	*Vitex cymosa* - flowers - Dichloromethane	445.0	74.17														
13	*Vitex cymosa* - leaves - Dichloromethane	372.0	62.00	-													
12	*Vitex cymosa* - leaves - Methanol	223.0	37.17	+	-												
11	*Vitex cymosa* - branches - Methanol	489.0	81.50	-	-	+											
10	*Vitex cymosa* - flowers - Methanol	244.0	40.67	-	-	-	+										
9	*Vitex cymosa* - roots – Hydroalcoholic	287.0	47.83	+	-	-	+	-									
8	*Vitex cymosa* - leaves – Hydroalcoholic	305.5	50.92	-	-	-	+	-	-								
7	*Vitex cymosa* - branches - Aqueous	132.0	22.00	+	+	-	+	-	-	-							
6	*Vitex cymosa* - flower branches - Aqueous	132.0	22.00	+	+	-	+	-	-	-	-						
5	*Vitex cymosa* - flowers - Aqueous	155.5	25.92	+	+	-	+	-	-	-	-	-					
4	*Vitex cymosa* - leaves - Aqueous	288.0	48.00	-	-	-	+	-	-	-	-	-	-				
3	*Vitex cymosa* - branches - Aqueous	132.0	22.00	+	+	-	+	-	-	-	-	-	-	-			
2	*Eschweilera pedicellata* - branches - Aqueous	233.0	38.83	+	-	-	+	-	-	-	-	-	-	-	-		
1	*Eschweilera pedicellata* - leaves - Aqueous	132.0	22.00	+	+	-	+	-	-	-	-	-	-	-	-	-	

Ri: Rank sum; $\overline{R}$: average of the ranks; H- value calculated = 50.77; H- value with an adjustment for the number of ties: 113.89; H- critical value corresponding to a chi-square equal to α-value (0,05); df(k-1): 13; Q-critical value: 3.456; +:significant difference.

Furthermore, this analysis (Table 3) evidenced a negative trend of *V. cymosa* flowers and leaves dichloromethane extracts on the survival of adults, related to the low polarity of extracts, because less polar extracts resulted in better results than more polar ones, with the same plant structures (Table 3). The discussions about solvents used for extraction and their relationship with plant parts are controversial. Some authors suggest that polar solvents extract substances like sugars and tannins, with low insecticidal activity (Jaglan et al., 1997; Cunha et al., 2006), but Mohapatra et al. (1995) obtained results in the opposite direction. In the present study, the influence of solvent in conjunction with plant part varied with the combination of both, and for *V. cymosa* leaves and flowers dichloromethane extracts, the lower polarity of the solvent resulted in a better percentage of dead individuals. So, the importance of testing distinct extracts, combining different plant parts with solvents of different polarities, becomes evident.

Given the overall low mortality, it was only possible to estimate LC_{50} for *V. cymosa* branches methanol extract, which was equal to 4.5 mg/mL (CI = 1.844-11.124). Even with the better results by

ingestion, it was not possible to make inferences about dose-dependent extract lethality.

Among the extracts that were active by ingestion assay, only *V. cymosa* leaves dichloromethane extract did not result in the reduction of the number of emerged individuals in the F1 generation. The reduction of the number of individuals in F1 has been attributed to the insecticidal effect on parent individuals, and in some cases, the repellent effect has been considered a second agent of grain protection, and has been observed in several studies (Silva-Aguayo et al., 2005; Tapondjou et al., 2005; Asawalam et al., 2006; Arannilewa and Odeyemi, 2007).

It is clear from the present work that some extracts not reduced the number of individuals emerged in the F1 generation as expected, but even increased it (Table 2). This could be due to the lack of parental adult sex distinction in the ingestion assay or from a possible generations overlap, because the individuals counting was not made on each day, only at the end of the 49 days. On the other hand, *V. cymosa* leaves and flowers aqueous extracts, even not providing good results by ingestion, reduced the number of individuals in F1. This result would be unexpected if we only consider that the insecticidal effect affects the number of individuals emerged in F1, however, the ovicidal and larvicidal effects which were not assessed, could decrease the number of individuals emerged.

The results observed in the present study are initials, hence, it would be premature to infer that the extracts tested have a good potential as botanical insecticides or grain protectors against *S. zeamais*, because high concentrations were required to achieve those results. So, we suggest that if future trials are conducted with the same plant extracts, they should be conducted in association with chromatographic fractionation.

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