Effect of Java grass (*Cymbopogon winterianus* Jowitt) essential oil on fall armyworm *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera, Noctuidae)

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ABSTRACT. There are many compounds extracted from plants, called allelochemicals that affect insect biolology and behavior. Among these substances the terpenoids are efficient on inhibiting reproduction, feeding and larval development. This research, developed at the Universidade Estadual Paulista, Botucatu, evaluated the bioactivity of Java grass, *Cymbopogon winterianus* Jowitt (Poaceae) essential oil (rich in citronellal and citronellol) on *Spodoptera frugiperda* (J.E. Smith). Two bioassays were conducted. The first assay, corn leaves with 10 cm long and 4 cm wide were divided in 3 equal parts. One of the end part was dipped into solutions containing 1.0, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001% of Java grass essential oil diluted in 0.05% emulsifier Tween 20. The other end part was dipped into water with 0.05% of the emulsifier Tween 20. Four neonate larvae were released in the center third of the leaves. The second assay, Petri dishes surfaces were treated with 1.0, 0.5, 0.1, 0.05 % of Java grass essential oil solutions diluted in 0.05% emulsifier Tween 20. A blank treatment 0.05% emulsifier Tween 20 was also used. After dried, the surfaces received 10 new hatched larvae and were covered with a plastic sheet. The results showed that Java grass essential oil presents insecticide properties and repellency against *S. frugiperda* larvae.

Key words: citronella, *Spodoptera frugiperda*, natural insecticide, essencial oil.

RESUMO. Ação do óleo essencial de Citronela (*Cymbopogon winterianus* Jowitt) sobre a lagarta do cartucho do milho *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera - Noctuidae). Existem muitos compostos extraídos de plantas, denominados aleloquímicos, que alteram a biologia e o compotamento dos insetos. Entre essas substâncias os terpenóides são eficientes inibidores do crescimento larval, reprodução e alimentação. Esse trabalho, realizado na Universidade Estadual Paulista, Botucatu, Estado de São Paulo, procurou verificar a bioatividade do óleo essencial da citronela, *Cymbopogon winterianus* Jowitt (Poaceae) (rico em citronelal e citronelol) sobre *Spodoptera frugiperda*. Foram realizados dois bioensaios. No primeiro, folhas de milho com 10 cm de comprimento e 4 cm de largura foram divididas em 3 partes iguais. Uma das partes de uma das extremidades foi mergulhada em soluções contendo 1,0, 0,5, 0,1, 0,05, 0,01, 0,005 e 0,001% de óleo essencial de citronella com 0,05% do emulsificante Tween 20, a outra parte foi mergulhada em água com 0.05% desse emulsificante. Quatro larvas recém-eclodidas foram colocadas no centro de cada folha. No segundo ensaio, superfícies de vidro (placas de Petri) foram tratadas com soluções contendo 1,0, 0,5, 0,1, 0,05 % do óleo essencial de citronela e 0,05% do emulsificante Tween 20, mais um tratamento testemunha (água com 0,05% emulsificante). Após a secagem da superfície, foram colocadas em cada placa de vidro, 10 lagartas recém-eclodidas e cobertas com filme plástico. Os resultados mostraram que o óleo essencial de citronela apresenta ação inseticida e de repelência para lagartas de *S. frugiperda*.

Palavras-chave: citrolnela, *Spodoptera frugiperda*, inseticida natural, óleo essencial.

Introduction

There are many plant compounds being used to protect plants against insect attacks. According to Butterworth and Morgan (1968), mentioned by Govindachari *et al*. (1996), the discovery of azadirachtin, substance with high deterrent potential in grasshoppers,when administered in the dosage of 40 g per liter, motivated studies with other hundreds of vegetable compounds with insecticidal activity during the last decades.

Aerts and Mordue (1997), working with triterpenoids extracted from *Azadirachta indica,* verified high deterrent activity when tested *on Spodoptera littoralis* Boisduval larvae and on *Schistocerca gregaria* Forskal nymphs.

According to Pickett (1991), efficient insecticide was obtained from plants since pirethrina I (component with the highest insecticide activity), extracted from Chrysanthemum cinerariefolium.

Bloem *et al*. (1989) listed lots of substances that affect insects' biology and behavior. Saponins, extracted from various plants, showed to be efficient on inhibiting *Tribolium casteneum* Herbst larval growing. Terpenoids glycosids taken out from organ pipes inhibited the development of *Drosophila nigrospiracula* Patterson. Glycoalkaloids from potato really affected *Leptinotarsa decemlineata* Say feeding, as well as the survival of *Empoasca fabae* Harris nymphs. The tomatine glycoalkaloid from tomato plants has shown a potent inhibitor in the development of *Helicoverpa zea* Boddie larvae.

Lindroth and Peterson (1988) evaluated the effect of two classes of phenols (the most abundant group of allelochemicals) on the performance (e.g. food intake, growing rate) during the last instars of *Spodoptera eridania* Cramer. These components caused on treated larvae an increase in mortality and a decrease of food intake and digestibility resulting in reduction in the larval growing rate.

Ahmad *et al*. (1993) presented a wide review about plant substances that act as insecticides, fungicides and nematicides, mentioning their dosages, indications, application methods and biological targets.

Cowles *et al*. (1990) studying the deterrent effects of some cynamic derivatives and monoterpenoids like citronellol and citronellal in *Delia antiqua* Meigen, verified no oviposition with BR_{90} (concentration needed to cause 90% of deterrency on oviposition) of 0,88% and 3,7%, respectively. The contrast between citronellal and citronellol seems to indicate that the alcohol form shows more activity than the aldehydic one.

According to Khambay and O`Conor (1993) the identification key of insecticidal activities of these compounds is the availability of bioassays that requires "in vivo" analysis, sampling, application methods (contact or residual) and the decision about the level of compound efficiency. So *Spodoptera frugiperda* (J. E. Smith), was the used specie because of its economic importance and its well known biology and behavior, which would permit the understanding of the results obtained from the experimentation with Java grasss (*Cymbopogon winterianus* Jowitt) (Poaceae) essential oil, aiming to verify its insecticidal and repellency activities.

Material and methods

This research was conducted at the Universidade Estadual Paulista "Júlio de Mesquita Filho" , Departamento de Defesa Fitossanitária - Botucatu (SP). The assays were performed at 25 +/- 2 °C, RH 70% and 14 h photophase, consisting of *S. frugiperda* rearing on artificial diets (proposed by Nalin, 1991) with different concentrations of Java grass (*C. winterianus)* essential oil.

Initially*, S. frugiperda* eggs were obtained from Esalq-USP and Rhodia Agro Ltda laboratories rearing. From these eggs it was established a stock rearing to produce insects to conduct this research.

The Java grass, *C. winterianus,* was grown in field on São Manoel Experimental Farm and the essential oil was obtained from distillation of plants leaves. The extracted oil was chemically analyzed at the Instituto Agronômico de Campinas (IAC) and presented the following composition: citronellal (42,15%), citronellol (22,03%) and geraniol (16,44%), indicating that more than 80% of the oil had the constituents reported in the literature with insecticidal activity (Carrol, 1994).

The first assay corn leaves 10 cm long and 4 cm wide were marked into 3 equal parts. One end part was dipped into a solution, previously prepared with the following Java grass essential oil concentrations 1.0%, 0.5%, 0.1%, 0.05%, 0.01%, 0.005% and 0.001% plus 0.05% of emulsifier Tween 20. The other end part was dipped in blank treatment, water with 0.05% of Tween 20.

After the solution was drained, the leaves, were placed on a white paper sheet on a table to dry out the water. Four neonate larvae were placed on the center third of each leave. After 2, 15, 30 and 60 minutes of larval inoculation, their behavior was evaluated verifying which part of the leaf they preferred: the treated third with its correspondent concentration, the third in the opposite side of the leaf with blank treatment or the middle one with no treatment (where larvae were released). To avoid the light attractive effect on the larvae, the leaves were distributed following a randomized design on a table placed in the center of a 16 m^2 room, with white walls, exclusively illuminated with eight 40 Watts fluorescent lamps (daylight). Each treatment was repeated eight times.

The second assay glass surfaces (Petri dishes) were treated with 1.0%, 0.5%, 0.1%, 0.05% solutions of Java grass essential oil plus 0.05% of the emulsifier Tween 20. The check treatment received water with 0.05% of Tween 20 instead of oil. After dried, the Petri dishes (15 cm diameter) received 10 neonate larvae and were covered with a plastic sheet. Each treatment was repeated five times, following a randomized design, in a room with the same conditions as the first assay. After 90 minutes the mortality percentage was evaluated.

The 0,05% of the emulsifier Tween 20® (ICI Americas Inc.) added to the aqueous solutions was necessary due to the oil nature of this vegetable extract. Thus, all treatments received this emulsifier concentration, including the blank.

The concentration, time and condition (treated and no treated) data were submitted to factorial analysis (7 doses x 4 times x 2 conditions) with the respective interactions and the averages were compared by Tukey test at 5% of probability. The larval mortality data were correlated with doses of Java grass essential oil by linear regration analises

Results

In the assay where the neonate larvae were released on corn leaves that had one of its third treated with Java grass essential oil, the treated areas were avoided by the larvae, mainly in the treatments with higher concentrations.

A significantly lower larval number was observed on treated parts of leaf surface than on untreated one (Table 1). The number of arrested larvae on the treated parts of leaves with 0,001% of Java grass essential oil was significantly higher than the other treatments, indicating that the arrested behaviour was less affected by the lower concentrations.

During this experimental period, there was no variation in the arrested larvae number to the treated part of leaves surface, indicating the repellency occurred immediately after the first larval contact with citronella oil. During the observation period, on untreated part of leaf surface, it was verified differences in the larval number, indicating larval mobility. These larvae left the untreated part of leaf surface going to other parts except those which received the Java grass essential oil treatments, confirming its repellency (Table 1).

The assay where the neonate larvae were released on glass treated surface showed larval mortality ranging from 1.00 e 0.50% of essential oil solutions was significantly greater than the other two solutions (0.1 e 0.05 %) (Figure 1). The observed mortality to the lower concentrations was equivalent to the check treatment. The correlation between java grass essential oil concentration and larval mortality showed high significance (Figure 2).

Table 1. Number of living larvae on treated and non treated areas of corn leaves with Java Grass essential oil against *S. frugiperda* larvae. Temperature 25+/-2°C, RH 70% and Photophase 14 h

Concentration (%)	Time	Condition		Average Conc.x Time	Average of Concentr.	Average Time
		Treated	Untreated			
1.0	2 min	0.50^{4}	7.50^{123}	4.00 a		
	15 min	1.00^{4}	6.00^{123}	3.50 a		
	30 min	1.25^{4}	5.25^{123}	3.25 a		
	60 min	0.25^{4}	6.00^{123}	3.12 a		
Average		0.75 B b	6.18 A b		3.47 a	
0.5	2 min	0.00^{4}	7.75^{12}	3.87 a		
	15 min	0.00^{4}	6.75^{123}	3.37 a		
	30 min	1.00^{4}	6.25^{123}	3.62 a		
	60 min	0.00^{4}	6.50^{123}	3.25 a		
Average		0.25 B b	6.81 A ab		3.53 a	
0.1	2 min	0.50^{4}	7.25^{123}	3.87 a		
	15 min	0.50^{4}	6.75^{123}	3.62 a		
	30 min	0.50^{4}	7.00^{123}	3.75 a		
	60 min	0.00^{4}	6.25^{123}	3.12 a		
Average		0.37 B b	6.81 A ab		3.59 a	
0.05	2 min	0.25^{4}	7.75^{12}	4.00 a		
	15 min	1.00^{4}	7.00^{123}	4.00 a		
	30 min	0.50^{4}	7.00^{123}	3.75 a		
	60 min	0.00^{4}	7.25^{123}	3.62 a		
Average		0.43 B b	7.25 A a		3.84 a	
0.01	2 min	1.00^{4}	6.25^{123}	3.62 a		
	15 min	0.75^{4}	6.50^{123}	3.62 a		
	30 min	0.25^{4}	7.00^{123}	3.62 a		
	60 min	0.50^{4}	6.50^{123}	3.50 a		
Average		0.62 B b	6.56 A ab		3.59 a	
0.005	2 min	0.50^{4}	7.50^{123}	4.00 a		
	15 min	0.25^{4}	6.75^{123}	3.50 a		
	30 min	0.25^{4}	6.50^{123}	3.37 a		
	60 min	0.50^{4}	6.50^{123}	3.50 a		
Average		0.37 B b	6.81 A ab		3.59 a	
0.001	2 min	1.25^{4}	5.50^{123}	3.37 a		
	15 min	2.25^{4}	5.25^{123}	3.75 a		
	30 min	1.75^{4}	5.00^{1}	3.37 a		
	60 min	2.00^{4}	4.75^{12}	3.37a		
Average		1.81 B a	5.12 A c		3.47 a	
		Condition X Time				
	2 min	0.57 B a	7.07 A a			3.82 a
	15 min	0.82 B a	6.42 A ab			3.62 ab
	30 min	0.78 B a	6.28 A b			3.53 ab
	60 min	0.55 B a	6.25 A b			3.35 b
Average condition		0.66 B	6.50 A			

Capital letters compare the average on lines and small letters on columns; Numeric indexes compare the averages of treated and untreated, at different times, for each concentration; Averages followed by the same letters or numbers do not differ by Tukey test at 5% of probability

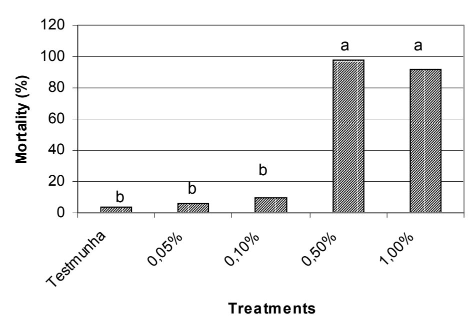

Figure 1. Larval mortality on glass treated surfaces. Temperature 25+/-2°C, RH 70%, and Photophase 14 h

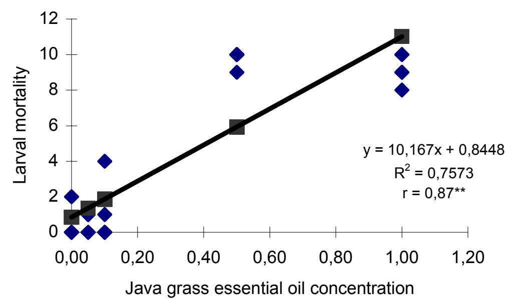

Figure 2. Correlation between java grass essential oil and larval mortality. Temperature 25+/-2°C, RH 70%, and Photophase 14 h

Discussion

Ouden *et al*. (1996) studying repellent effect of some plant substances to *Delia radicum* Linneaus detected aldehyde citronellal did not exhibit the repellency effect as expected and the reason could be its volatilization.

As larvae were released on the Petri dishes immediately after they became dried and they were covered with a plastic film. It was not possible to distinguish the contact effect from the effect caused by the volatilization of the essential oil. In the first assay there was not volatilization effects due to the short period of evaluation.

The results indicated no relationship between the concentrations and mortality (Figure 1) which was not observed in great part of the assays discussed by Labinas (1998), who studied the effects of Java grass essential oil added to artificial diets used to study the *S. frugiperda* biology and behavior. As citronella oil is a natural substance, several problems occurred during the manipulation process, which explains the reason that Labinas (1998) did not find any relation between concentration and mortality.

The monoterpenoid structure characteristics that constitute Java grass essential oil (*C. winterianus*) may influence its insecticide properties. The saturation level and the functional group present in the molecules influence the penetration of these substances through insect cuticule, may affect the movement and the interactions between these and other molecules, as well as their degradation. Besides the unknown way of action, acute, sub-acute and lethal effects have been discussed. The results show that monoterpenoids can act as insecticide but their action greatly vary and all structure modification in the molecule can result in big differences in their toxicity (Rice and Coats, 1994).

Concluding, the Java grass essential oil shows repellency and insecticide properties to *S. frugiperda* larvae. The total mortality of *S. frugiperda* larvae occurred next to the concentrations of 1.0 %.

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