Toxicity of essential oils to the larvae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Neiva Knaak*, Shana L.F. Wiest, Tiago F. Andreis and Lidia M. Fiuza

ABSTRACT

This study evaluates the effect of plant oils on the caterpillars of *Spodoptera frugiperda*. The oil obtained from *Cymbopogon citratus* had the lowest median lethal concentration (LC_{50}=0.19 µL/cm²) in comparison with the other treatments. The contact test demonstrated that *Zengiber officinale* was the most efficient with a median lethal dose **(**LD_{50}) of 0.55 µL/larvae. In the repellency tests, the caterpillars showed a for rice leaves treated with 0.1 and 1% of the oil of *Artemisia absinthium*, 24 and 48 hrs after the application treatment. The treatments with the oils of *Mentha* sp., *Tanacetum vulgare*, *Ruta graveolens* and *Z. officinale* at 0.1% did not differ from the control. Thus, among the essential oils studied in this work, the most promising for the control of larvae of *S. frugiperda* were *Z. officinale* and *C. citratus*, the former being more efficient.

Key words: Biossays, carterpillar, essential oils, insecticidal effect.

INTRODUCTION

Insects are among the principle agents that prejudice cultivations and can cause losses up to 37% of the crops (Falco and Silva-Filho, 2003). Among these, *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), known as the fall armyworm (Martins and Afonso, 2007) is a polyphagous pest that may attack 23 families of plants cultivated in Brazil (Cruz *et al*., 1999), such as cotton, alfalfa, peanuts, rice, oats, potato, sweet potatoes, sugarcane, vegetables, corn, soya and wheat, and is most commonly found in the grasses (Polanczyk and Alves, 2005). It can reach high population levels when the climatic conditions favor its biology, such as in areas of high temperatures and low rainfall (Martins and Afonso, 2007).

Spodoptera frugiperda is usually controlled by the use of synthetic insecticides now widely available at low cost but which have the disadvantage of leaving residual traces in the food and in the environment as well as causing intoxication in the producers/consumers, creating resistant pest populations and other problems (Viana and Prates, 2003). Because of these consequential effects of the synthetic insecticides, the search for cleaner technologies is being intensified with emphasis on insecticides derived from plants (Tavares and Vendramim, 2005).

The use of substances extracted from plants as insecticides has many advantages as compared to synthetic preparations for pest control because the plant material is renewable and degrades rapidly that is, it does not persist in the environment. Furthermore, the plant insecticide is less likely to affect beneficent or non-targeted organisms (Aguiar-Menezes, 2005), and presents less risk that the pests will develop resistance to it because the insects development of such resistance to these substances which are composed of associations of various active substances is a slow process (Prates *et al*., 2003).

The insecticidal activity of the many plant extracts and essential oils has been proven by various researchers (Breuer and Schmidt, 1995; Hiremath *et al*., 1997; Zhao *et al*., 1998; Isman *et al*., 2001; Muthukrishnan and Pushpalatha, 2001; Wheeler and Isman, 2001; Sadek, 2003; Pavela, 2005; Kordali *et al*., 2006; Fazolin *et al*., 2007; Silva *et al*., 2009; Lima *et al*., 2009a).

In this context, the present work aims to evaluate the insecticidal effect of the essential oils *Zingiber officinale* Roscoe (Zingiberaceae), *Malva* sp. L. (Malvaceae), *Artemisia absinthium* L. (Asteraceae), *Tanacetum vulgare* L. (Asteraceae), *Cymbopogon citratus* (DC) Stapf. (Poaceae), *Ruta graveolens* L. (Rutaceae) and *Mentha* sp. L. (Lamiaceae) on the first instar caterpillars of *S. frugiperda*.

MATERIALAND METHODS

The *S. frugiperda* caterpillars were collected in cultivations of irrigated rice in the State of Rio Grande do Sul, Brazil, and maintained on a Poitout and Bues (1970) diet in the Insect Breeding Room of the *Laboratório de Microbiologia e Toxicologia/Unisinos.* The biological cycle was developed under controlled conditions (25 ± 2°C, photo period of 12 hrs at 70% RH).

The medicinal plants (*Z. officinale*, *Malva* sp., *A. absinthium*, *T. vulgare*, *C. citratus*, *R. graveolens* and *Mentha* sp.) were processed by the hydro-distillation method in adapted Clevenger equipment (Clevenger, 1928). The oils were stored in hermetically sealed, amber-colored glass recipients at 4ºC. The identification of the chemical components of essential oils was carried out by gas chromatography followed by mass spectrometry (GC/MS). The oil constituents were identified by combining the mass spectrum and linear retention rates (LIR's) using source of standard solution C9 to C26 hydrocarbons. The GC analysis was performed on a Hewlett Packard 6890 gas chromatograph equipped with an HP-data processor Chemstation, using apolar HP-Innowax column (30 m x 320 mm id) 0.50 mm film thickness (Hewlett Packard, USA) with the following temperature program: 40°C (8 min) to 180°C at 3°C/min, 180-230°C at 20°C/min, 230°C (20 min), injector temperature 250°C, split ratio 1:50, FID detector temperature 250°C; gas H2 carrier (34Kpa), volume injected 1 mL diluted in hexane (1:10). All analyses were performed by the technical staff of the Laboratory of Essential Oils the *Universidade de Caxias do Sul*.

The essential oils were biotested on first instar *S. frugiperda* caterpillars using five concentrations selected in preliminary tests. In the treatments, we applied 10 µL of each concentration on sections of rice leaves of 1 cm diameter arranged on acrylic mini-plates containing moistened filter paper, on which 50 caterpillars were individualized per treatment. For the control, the treatment material was substituted by acetone. Each experiment consisted of six treatments including the control group: *Z. officinale* (0.2; 0.5; 2, 3.5; 4.5%), *C. citratus* (0.05; 0.1; 1; 2.5; 3.5%), *T. vulgare* (0.2; 0.6; 2; 3.5; 5%), *Mentha* sp. (0.1; 0.5; 1; 3.6; 5%), *R. graveolens* (1; 2; 3.5; 4.5; 5%), *Malva* sp. (1; 2; 3; 5; 6%), *A. absinthium* (2; 3; 5; 10 and 18%) and three repetitions, totalizing 750 caterpillars for each essential oil evaluated. The treatments were maintained in chambers at 25ºC, and photo phase of 12 hrs. The mortality was evaluated up to the 7 day after the treatments, and subsequently corrected by the Abbott (1925) formula. The median lethal concentration (LC_{50}), of each essential oil was determined by a Probit Analysis (Finney, 1971).

For the tests of the effects of topical application, the material was applied to the abdominal segments of the caterpillars of the second instar of *S. frugiperda*, in volumes of 0.1, 0.5 and 0.8 µL of the essentials diluted in 2 µL of acetone. The larval mortality was evaluated for 24 hrs after the application of the treatment totalizing 210 caterpillars per essential oil tested. The mortality values were corrected by the Abbott (1925) formula. The median lethal dose (LD_{50}) of each essential oil for each target species was calculated by the Probit Analysis Method (Finney, 1971).

For the tests of the repellence, essentials oils were diluted in acetone at 0.1 and 1% (v/v), and maintained at 4°C in an amber flask while the experiments were being prepared. The tests were performed on Petri disks of 19 cm in diameter on which two fragments of rice leaves (5 cm in length) were distributed. These fragments had been previously disinfected with hypochlorite at 10% and treated with the essentials in concentrations of 0.1 and 1% (v/v) and another where the product was substituted by acetone. The treated leaves were distributed equally on the plates in the form of an arena, and, in the center, 20 caterpillars of the first instar of *S. frugiperda*, were released. The plates were then placed in a climate controlled chamber kept at 25 ± 2°C, with relative humidity of 70 ± 10% and photo-phase of 12 hrs. The observations were based on two countings of the caterpillars found on each leaf segment after 24 and 48 hrs (Castro *et al*., 2006). The tests were realized in triplicate and with three repetitions in the time-scale, totalizing 180 caterpillars evaluated per concentration of the each essential oil tested. The measurements of the percentages of repellence were made using the formula PR = (NC - NT)/(NC + NT) x 100 (Obeng-Ofori, 1995), where PR equals the average percentage of repellence; NC, the total of insects attracted to the control; and NT, the total of insects attracted to each treatment with the essential. The data was transformed into x+0.5 and submitted to Variance Analysis, and the measurements were compared by the Tukey method using a probability of 5%.

RESULTS

The results of the determination of the LC_{50} of the essential oils of *R. graveolens*, *Malva* sp., *A. absinthium*, *Z. officinale*, *Mentha* sp., *C. citratus* and *T. vulgare* (Table 1) applied *via oral* to the caterpillars of the first instar of *S. frugiperda*, showed that the *C. citratus* oil produced the lowest LC_{50} (0.19 µL/cm²) when compared with the other treatments.

The results of the contact test (Table 2) after the topical application of the essential oils to caterpillars of the 2º instar of *S. frugiperda*, was evaluated 24 hrs after the application of the treatments, and showed the *Z. officinale* oil as the most efficient with an LD_{50} de 0.55µL/caterpillar.

In the analysis chemical composition of the essential oils of *T. vulgare* (93.77% alpha-thujone), *R. graveolens* (41,07% of nonanone and 45,89% undecanone), *Malva* sp. (14,91% para-

Table 1. Lethal concentration (LC_{50}) and median lethal dose (LD_{50}) of treatment by ingestion and topical application of essential oils of medicinal plants for caterpillars of *Spodoptera frugiperda* (Lepidoptera, Noctuidae).

Treatments	LC_{50}			LD_{50}		
	(µL cm⁻²) n=540	CI (Li-Ls)*	χ^2	(µL) n=270	CI (Li-Ls)*	χ^2
Zingiber officinale	0,25	0,20-0,35	0,09	0,55	0,49-0,62	1,43
Cymbopogon citratus	0,19	0,13-0,38	2,05	2,36	1,66-5,18	17,68
Artemisia absinthium	2,09	1,64-2,96	0,49	5,51	4,14-8,36	1,49
Ruta graveolens	0,62	0,49-1,02	4,31	1,22	0,94-1,98	4,25
Malva sp.	0,67	0,58-0,82	0,57	1,07	0,98-1,21	1,09
Mentha sp.	0,33	0,16-1,93	4,21	4,16	2,57-10,96	3,41

*CI – confidence interval, calculated at 90% probability by Probit Analysis; Li=lower limit; Ls=upper limit.

mentone, 19,55% carvone and 38,78% citronellol), *A. absinthium* (40,61% alpha-thujone), *Z. officinale* (15,95% geranial and 13,27% alpha-zingibereno), *C. citratus* (47,53% geranial and 31,50% neral) and *Mentha* sp. (91,49% neral) identified by gas chromatography, were found as major components.

The results of the repellence tests with essential oils on the caterpillars of the first instar of *S. frugiperda* (Table 2), shows that they prefer rice leaves treated with 0.1 and 1% of *A. absinthium* oil 24 and 48 hrs after the application of the treatments (HAAT). The data on treatment with the *Mentha* sp., *T. vulgare*, *R. graveolens* and *Z. officinale* oils at 0.1% did not differ from the control ($p>0.05$), 24 and 48 hrs after the application of the treatments (Table 2). With an increase in the concentration to 1%, the essential oils tested, except for *A. absinthium* and *R. graveolens*, presented repellent effects 24 and 48 HAAT.

DISCUSSION

This data of LC_{50} the greater effectiveness of the essential oils in comparison with the plant extracts which were not lethal to *S. frugiperda*. Comparing this data with other studies of essential oils it may be mentioned that Tapondjou *et al.* (2005), evaluating the essentials of *Eucalyptus saligna* Smith (Myrtaceae) and *Cupressus sempervirens* L. (Cupressaceae), considered promising the result of LC_{50} - 0.36 and 0.84 µL/cm² of oil respectively obtained for *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Because of this, one can state that the insecticide potential of the medicinal plant essential oils of this study (Table 1) on the lepidopteran target was more efficient, except for *A. absinthium,* than that presented by the authors cited. In the present study, all the indices of toxicity (LD_{50}) from the topic application were superior to those of the ingestion applications, demonstrating that the action of the essential oil evaluated in this study is associated to the ingestion of the treatment by the target insect, the fall armyworm.

Similarly, Fazolin *et al*. (2005) found that the toxicity of the *Piper aduncum* L. (Piperaceae) oil, rich in dilapiol, for *Ceratoma tingomarianus* Bechyné (Coleoptera: Scarabaeidae) was high, with a LC_{50} of 0.06 µL/cm⁻² and LD_{50} of 0.002 µL/mg⁻. Pavela (2005) found that twenty-three oils were highly toxic after topical application to *S. littoralis* (Lepidoptera: Noctuidae), and that in eight of these: *Mentha citrate* (Ehrh.) (Lamiaceae), *Salvia sclarea* L. (Lamiaceae), *Nepeta cataria* L. (Lamiaceae), *Origanum vulgare* L. (Lamiaceae), *O. compactum* Benth. (Lamiaceae), *Melissa officinalis* L. (Lamiaceae), *Thymus mastichina* L. (Lamiaceae) and *Lavandula angustifolia* L. (Lamiaceae) the LD_{50} 0.05 µL/larva was observed – that is, lower values than in this present research.

In evaluating the data repellency, this essential oils of *Artemisia absinthium* attracts *S. frugiperda* ($PR<0$). Corroborating the findings of this study, Peres *et al*. (2009), verified that *Tagetes patula* L. Asteraceae) plant is also attractive to Thysanoptera: *Neohydatothrips* sp., *Frankliniella* sp., *F. schultzei* and *Caliothrips* sp. Thus, *A. absinthium* can be recommended for planting along the margins of crops to reduce infestation by phytophagous species. It can be assumed that the attraction exercised by the plant can be attributed to the presence of secondary metabolic substances that act as analogies of the juvenile hormones of the insects (Aguiar-Menezes, 2005).

The *R. graveolens* essential oil (at 1%) caused mortality of 100% to the caterpillars of *S. frugiperda*, 24 hrs after the application of the treatments (HAAT). In this study, we observed that as we increased the concentration - independently of the plant species utilized - the percentage of repellence increased in view of the fact that the repellent effect was accentuated with the quantity of the bioactive substances present in the essential oils.

The success of the search for a product of natural origin depends of the availability of the plant species, detection of the bioactive products, as well as the chemical synthesis of the active ingredients and the clarification of the target site of the insecticide molecule. Based on the observations it is concluded that, of the essential oils studied in this work, the most promising plant oil for the control of the caterpillars of *S. frugiperda* are that of *Z. officinale* and *C. citratus*, and that *Z. officinale* is the more efficient of the two.

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