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Research Article

Effects of geraniol and citronellal compounds on biochemical and reproductive parameters of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract: The essential oils of plants are composed of several volatile compounds, which may have different concentrations and may be determinant for their toxicity. Thus, in this work, sublethal and lethal effects of the compounds, geraniol and citronellal on the biochemical and reproductive parameters of *Spodoptera frugiperda* were investigated. For the geraniol compound the LD_{30} of 9.42 mg/g and LD_{50} of 13.65 mg/g was used, while for the citronellal LD_{30} of 0.06 mg/g and LD_{50} of 0.08 mg/g. Pure acetone was used in the control. Third instar caterpillars were treated topically in the prothoracic region by applying 1 µl of the respective compounds with a Hamilton™ 50 µl syringe. After 48 h the caterpillars were macerated in sodium phosphate buffer at a ratio of 4 caterpillars / 5 ml of the buffer, the levels of total protein, total sugar, lipid and glycogen were measured. The daily posture was evaluated in order to determine the periods of pre-oviposition, oviposition, post-oviposition and egg quantity. The results showed a reduction in the concentration of proteins and sugars for both compounds and concentrations studied. There was no change in lipid concentration. Citronellal increased the concentration of glycogen for both concentrations. The oviposition period and the number of eggs were reduced. However, there was no difference for the pre-oviposition and post-oviposition periods. Thus, it is inferred that geraniol and citronellal compounds cause alterations in the biochemical parameters that reflect in the reproduction of *S. frugiperda*.

Keywords: armyworm, isolated compounds, nutritional constituents, reproduction

Introduction

The high corn yield can be obtained by efficiently controlling its main pest, *Spodoptera frugiperda* (J.E. Smith). However, in Brazil the predominance of synthetic insecticides is still recurrent, which are used without taking into account the principles of Integrated Pest Management. Thus, the adoption of insecticides with low environmental impact, such as insecticidal plants, is fundamental to the success of pest control (Diéz-Rodrigues and Omoto, 2001).

One of the classes of plant-derived compounds, which has been prominent in insect

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control, are the essential oils, which are already part of formulations of pesticides, capable of killing and repelling insects (Isman, 2000). Some essential oils have already been evaluated for *S. frugiperda*, such as citronella, *Cymbopogon winterianus* Jowitt causing food deterrence and mortality (Labinas and Crocomo 2002). Extracts from other species such as *Foeniculum vulgare* (Mill.), *Ocimum gratissimum* L. and *Eucalyptus staigeriana* F. Muell., in sublethal doses interfered in biological parameters and *O. gratissimum* also altered reproductive parameters (Cruz *et al*., 2017).

The essential oils are composed of several volatile compounds, which may have different concentrations and may be determinant for their toxicity (Jemâa *et al*., 2012). Gusmão *et al*. (2013) and Silva *et al*. (2016), through chromatographic analyzes coupled to mass spectrometry, verified that the oil of *C. winterianus* presents the main constituents of geraniol and citronellal. These chemical compounds were also found by Cruz *el al*., (2016) in *O. gratissimum* oil, in addition citronellal was also present in the oils of *F. vulgare*, *E. staigeriana* and *E. citriodora*. Geraniol and citronellal are terpene compounds that have been shown to be of agricultural interest and can act as repellents, insecticides and growth inhibitors (Isman, 2000; Hummelbrunner and Isman, 2001; Isman, 2006; Chen and Viljoen, 2010).

Studies that address the interference of isolated chemical compounds on biochemical parameters are relevant, since researches have shown that botanical insecticides may interfere with the absorption and metabolism of nutrient constituents of insects (Sharma *et al*., 2011). Changes that occur in this sense can have negative consequences on the biological cycle of insects. Silva *et al*. (2016) verified that the *C. winterianus* oil in sublethal dose affects the biochemical profile of *S. frugiperda* caterpillars, causes reduction of proteins, lipids and total sugars, and increase of glycogen.

In the case of isolated or associated chemical compounds, Cruz *et al*. (2017) found that the chemical compounds limonene, trans-anethole and limonene + trans-anethole reduced the amount of lipids, protein, total sugars and glycogen, showing greater expression when the compounds were associated, except glycogen. Therefore, chemical compounds of important essential oils in the control of *S. frugiperda* can be explored for a better elucidation of their effects on the pest. Thus, the present study aimed to evaluate the sublethal and lethal effects of geraniol and citronellal on biochemical and reproductive parameters of *S. frugiperda*.

Materials and Methods

The present research was developed in the Laboratory of Histology of the Department of Morphology and Animal Physiology, Federal Rural University of Pernambuco (UFRPE).

Obtaining and breeding insects

S. frugiperda caterpillars were obtained from the stock of the Agricultural Entomology Laboratory of the Department of Agronomy of the Federal Rural University of Pernambuco (UFRPE), maintained at a temperature of 25.2 ± 1.4 ºC, relative humidity of 67 ± 0.7% and photophase of 12 h, and fed with modified Greene artificial diet, recommended for the species (Busato *et al*., 2006).

Obtaining Chemical Constituents

The chemical compounds were obtained from Sigma-Aldrich® with purity of 99%.

Extraction and Quantification of Total Soluble Proteins

For the experiments, third instar caterpillars (10 days old) were used, with an average weight of 78mg. The treatments consisted of: dilution of acetone compounds in LD_{30} and LD_{50} previously estimated by Cruz *et al*. (2016). For the geraniol compound the LD_{30} of 9.42 mg/g (7.38-10.92) and LD_{50} of 13.65 (11.34-15.97) mg/g were used, while for the citronellal the LD_{30} 0.06 (0.05-0.07) mg/g and LD_{50} of 0.08 (0.07-0.08) mg/g. Pure acetone was used in the control. In the prothoracic region 1.0 µl of the respective compounds were applied with the aid of a Hamilton™ 50 µl syringe. The caterpillars were individualized in flat bottom glass tubes and sealed with plastic film. Artificial diet was used for feeding. Each sample was composed of four caterpillars, and for each treatment 10 samples were obtained, totaling 40 caterpillars/treatment. The method of Bradford (1976) was used to determine the soluble proteins. The processing occurred after 48h of the installation of the experiments. The caterpillars were immobilized at 40 °C and then macerated in sodium phosphate buffer (pH 7.4 and 0.1 M) in the ratio of four caterpillars / 5 ml of the buffer. 1.0 ml of the mixture (caterpillars + buffer) was pipetted and stored in a properly labeled microtubule. The procedure was carried out at low temperature to avoid oxidation of the samples. These were centrifuged for 3 minutes at 3000rpm. After centrifugation, 100 µl of each sample was taken and placed in glass tubes and 5 ml of Bradford dye (0.01% Comassie Blue G-250, 8.5% phosphoric acid and 4.7% ethanol). The tubes were homogenized in a vortex type stirrer and were allowed to stand for 2 min. The spectrophotometer was then read at a wavelength of 595 nm. The unit used was µg/mL. The results were submitted to ANOVA and the means were compared by the tukey test at the 5% probability level using SAS Proc GLM (SAS Institute 2001).

Analyses of lipid, total sugar and glycogen

3rd instar larvae were macerated in sodium phosphate buffer (pH 7.4 and 0.1 M) at a ratio of 4 caterpillars / 5 ml of the buffer. Each sample consisted of four caterpillars and for each treatment 10 samples were obtained, totaling 40 caterpillars/treatment. The content of lipid, total sugar and glycogen were evaluated using the Van Handel method (Van Handel, 1985a,b), where 200 µl of the homogenate was added to 200 µl of sodium sulfate plus 800 µl of methanol and chloroform (1: 1), And centrifuged at 2000 rpm for 2 min. The precipitate was used for the glycogen analysis, and the supernatant was transferred to a test tube where the lipid and sugar were separated. The lipid was analyzed spectrophotometrically, using the phosphoric-vanillin acid method, whereas for the total sugar and glycogen, the sulfuric-anthronic acid method was used. The absorbance was read at 625 nm. The results of the lipid, sugar and glycogen contents were submitted to ANOVA and the means were compared by the tukey test at the 5% probability level using SAS ProcGLM (SAS Institute 2001).

Effect of compounds on reproduction of *S. frugiperda*

Third instars were subjected to the LDs 30 and 50 of the geraniol and citronellal compounds through topical contact. Then they were individualized in flat bottom glass tubes, fed daily with specific artificial diet until pupae formation. These were sexed and after adult emergence, couples were formed, two per cage with five replicates per treatment. The couples were housed in a PVC cage with dimensions of 10 cm x 15 cm (diameter and height) coated internally with sulphite paper, as a substrate for oviposition. The moths were fed with 10% honey solution and kept in an air-conditioned room. All experiments were performed at 25.2 ± 1.4 ° C, 67 ± 0.7% RH and 12 h photophase. The eggs laid were collected daily, counted and conditioned in Petri dishes with dimensions of 10 cm in diameter and maintained in the same conditions as previously described. Finally, the period of pre-oviposition, oviposition, total number of eggs and post-oviposition were evaluated. The analyses were submitted to the tukey test at 5% probability by SAS (SAS Institute 2001). The data were transformed into $(x + 1)^{1/2}$. Through confirmed mortality data the average survival rate was determined.

Results

Quantification of total soluble proteins

Biochemical tests for the extraction and quantification of total soluble proteins showed that the third instar larvae of *S. frugiperda* treated with LD_{30} and LD_{50} of the geraniol and citronellal compounds showed a reduction in their content (Fig. 1).

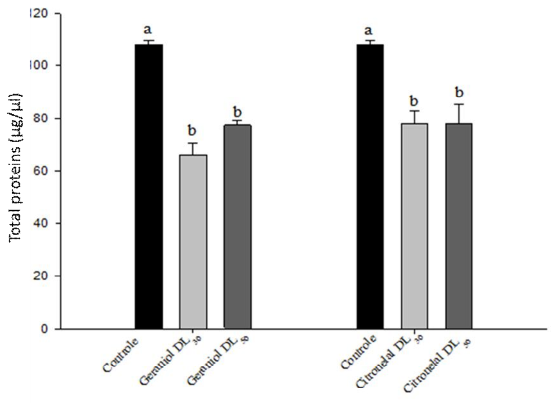

Figure 1 Amount of total proteins from *Spodoptera frugiperda* 3rd instar caterpillars subjected to LD_{30} and LD_{50}, by topical contact of the geraniol and citronellal compounds.

Analysis of lipid, total sugar and glycogen

Neither of the two compounds northe doses used affecred the amount of lipid compared to the control (Fig. 2). For the analysis of total sugar, there was a significant reduction in the caterpillars of both treatments and concentrations (Fig. 3). Geraniol at LD_{30} did not affect the glycogen content, whereas at LD_{50} it induced its reduction. However, the citronellal compound at both doses caused an increase of glycoen (Fig. 4).

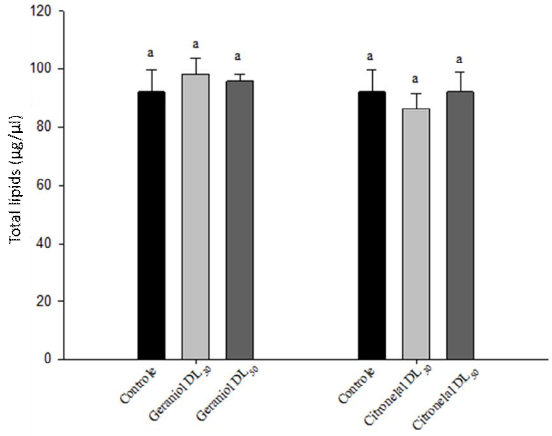

Figure 2 Quantity of total lipids derived from *Spodoptera frugiperda* 3rd instar caterpillars subjected to LD_{30} and LD_{50}, by topical contact of the geraniol and citronellal compounds.

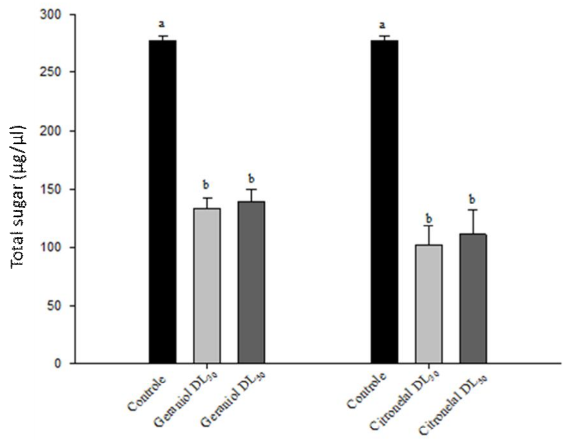

Figure 3 Amount of total sugar from *Spodoptera frugiperda* 3rd instar caterpillars submitted to LD_{30} and LD_{50}, by topical contact of the geraniol and citronellal compounds.

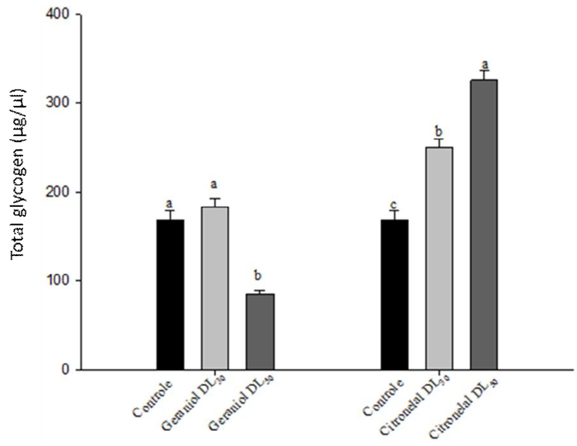

Figure 4 Quantity of total glycogen from third instar caterpillars of *Spodoptera frugiperda* submitted to LD_{30} and LD_{50}, by topical contact of the geraniol and citronellal compounds.

Effect of compounds on reproduction of *S. frugiperda*

The geraniol and citronellal compounds at LD_{30} and LD_{50} caused a significant reduction in the oviposition period and in the amount of eggs of adults from 3rd instar caterpillars treated by topical contact. However, there was no change in the pre-oviposition and post-oviposition periods (Table 1).

Table 1 Pre-oviposition period, oviposition, post-oviposition period and total number of eggs of two *Spodoptera frugiperda* females treated as larvae with LD_{30} and LD_{50} of the geraniol and citronellal compounds. Temperature: 25.2 ± 1.4 °C. Humidity: 67 ± 0.7% and 12 h photophase.

Treatments	Pre-oviposition (days)[1]	Oviposition (days)[1]	Post-oviposition (days)[1]	Number of eggs Per cage [1]
Control	2.40 ± 0.24a	7.60 ± 0.24a	2.60 ± 0.24a	2170.80 ± 197.02a
Geraniol LD_{30}	3.60 ± 0.24a	5.00 ± 0.55b	2.20 ± 0.20a	704.20 ± 171.02b
Geraniol LD_{50}	3.00 ± 0.32a	3.80 ± 0.37b	2.80 ± 0.86a	729.00 ± 257.02b
Citronellal LD_{30}	2.60 ± 0.40a	3.40 ± 0.60b	3.60 ± 0.98a	598.40 ± 86.51b
Citronellal LD_{50}	3.00 ± 0.32a	4.20 ± 0.37b	2.20 ± 0.58a	527.00 ± 88.95b

[1] Original data for analysis were transformed into $(x + 1)^{½}$, Means followed by the same letter in the columns do not differ significantly by the Tukey's test ($P < 0.05$).

Discussion

Geraniol and citronelal caused reduction in some biochemical parameters, such as protein and sugars. According to Sharma *et al.* (2011) and Yazdani *et al.* (2013) nutritional parameters may suffer interference caused by botanical insecticides, a fact that is in line with the results of the present research. Proteins participate in innumerable processes related to insect survival, and alterations in protein levels can cause negative reproductive effects, as they directly affect vitellogenesis (Panizzi and Parra, 2009; Guizzo *et al.*, 2012; Rosas-Mejía *et al.*, 2015). As verified in the research in question according to Senthilkumar *et al.* (2009) protein reduction is probably due to interference caused by compounds present in botanical insecticides that act on the hormones that regulate protein synthesis. Many of the insecticides have deterrent properties and reduce feed efficiency in insects, which in turn reduces some vital components like proteins in the body. The compounds may have inhibited the feeding of the larvae and as a result the protein decrease could be due to hunger stress (Etebari *et al.*, 2006).

The reduction in carbohydrate content may compromise insect survival (Parra, 1999), because these constituents function as an energy source, besides being responsible for innumerable metabolic and structural functions, and in the formation of chitin; Can be converted into lipids, as well as participate in amino acid synthesis (Chapman, 2013; Arrese *et al.*, 2010).

In insects, lipids play important roles, are constituents of cellular structures, act as hormones and form important energy reserves of great metabolic activity such as flight and egg production (Arrese *et al.*, 2001). Although the results obtained in this research did not present negative changes for the lipids in either of the compounds and concentrations studied; oviposition and fecundity showed a significant reduction. These results may suggest that lipids were not metabolized and/or absorbed adequately for egg production in *S. frugiperda.*

It can be inferred, therefore, that compounds of essential oils have an effect on the nutritional indexes of treated larvae. This suggests a reduction in the efficiency of using ingested foods when these products are used (Etebari *et al.*, 2006).

In general, studies related to biochemical alterations are performed with extracts or essential oils, such as extracts of *Eucalyptus globulus* Labill. *Cymbopogan citratus* (DC.) Stapf, *Artemisia annua* L., *Gendarussian justice* Burm F., *Myristica fragrans* Houtt, *Annona squamosa* L., and *Centella asiatica*, which reduced rates of protein, carbohydrate, lipid and some amino acids of larvae and adults of *Anopheles stephensi* (Senthilkumar *et al.*, 2009).

According to tests performed by Nasr *et al.* (2017) the essential oil of *Origanum vulgare* L. significantly reduced the total protein and triglycerides of third instar larvae of *Plutella xylostella* L., in sublethal and lethal doses, corroborating with several other studies that point out the toxic properties of insect oils - Prague. These authors suggest that in physiological studies, determination of the total protein and of many chemical macromolecules such as lipids and carbohydrates are very important. Etebari *et al.* (2006) reiterated that many products have anti-feedant properties and reduce the efficiency of insect feeding, which in turn reduces some vital components, such as proteins.

As regards effect of components of essential oils on the aforementioned macromolecules, Cruz *et al.* (2017) reported decreases in proteins, lipids, sugars and glycogen in *S. frugiperda* caterpillars when treated with limonene and trans-anethole compounds, and these results were more significant when the compounds were used in combination. In addition, the experiments showed different relationships with glycogen levels, since the citronellal compound caused an increase of glycogencorroborating with data obtained by Silva *et al.* (2016), who also observed increase of glycogen caused by *C. winterianus* oil. These authors suggested that the increase was caused by the citronellal, the major compound present in this oil which is the object of our study as well.

Regarding the reproductive aspects, it is possible to state that the geraniol and citronellal compounds had a negative effect on the oviposition and fecundity of *S. frugiperda*, confirming the correlation that the reproductive success of the insects is related to the nutrients acquired in the immature phase (Sousa *et al.*, 2015), since nutritional resources are acquired to ensure growth and reproduction. The vitellogenesis and maturation of ovules to occur satisfactorily depends on the energy resources that have been acquired. A reduction in reproduction shows that the compounds may have influenced the non-metabolization of most nutritional constituents or inefficiently acquired in the larval period (Attardo *et al.*, 2005; Milano *et al.*, 2010; Etebari *et al.*, 2006).

Other studies deal with the effects of compounds and essential oils on reproductive aspects such as Sharab *et al.* (2009), who verified that the compound eugenol and the oil of *Mentha piperita* L., each at concentration of 0.01% caused significant reduction in the fecundity of *Phthorimaea operculella* Zell. The same authors also tested leaves, fruits or seeds of 14 plants for their essential oils and verified that the oils of *Allium cepa* L., *Curcuma longa* L., *Clolocasia antiquorum* Schott, *Ocimum basilicum* L., *Dodonaea viscose* (L.) Jacq. and *Thuja orientalis* L. caused a reduction in egg deposition. (+) - fenchone and (-) - β - pinene significantly reduced the oviposition potential of *Pseudaletia uninpucta* Haworth females by 46% and 33% (Sousa *et al.*, 2015).

The present study showed efficacy with respect to the isolated compounds, whereas Hummelbrunner and Isman (2001) found that isolated compounds were less effective than conventional insecticides. On the other hand, when mixtures of some compounds were tested, they proved to be more effective than botanical insecticides such as rotenone and neem. These same authors verified that a mixture of R-terpineol, eugenol and thyme oil was 300 times less toxic to fish than azadirachtin, rotenone and pyrethrum. This can be attributed to detoxification pathways and mode of action of monoterpenoids (Koul *et al.* 2013). Several compounds of essential oils have been reported as octopamine blockers, which is a unique neurotransmitter for insects, and which is also why they are less toxic to vertebrates (Hummelbrunner and Isman, 2001). Compounds of essential oils may be considered safer for the environment than other plant-derived chemicals, such as azadirachtin, rotenone, or pyrethrum (Stroh *et al.*, 1998). In general, it is believed that essential oils by having a large number of compounds and thereby increasing the spectrum of insecticidal action are more effective than pure compounds. However, there are controversies regarding the efficacy of essential oil compounds, since different species have different responses to individual compounds.

Conclusions

It can be concluded from the present study that the geraniol and citronellal compounds cause reduction in biochemical and reproductive parameters, and thus can be used in pest control in ecologically based integrated management programs. In addition, the identification of essential oil compounds may allow the development of more effective control agents.

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اثرات ترکیبات ژرانیول و سیترونلال روی پارامترهای بیوشیمیایی و تولید مثلی *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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چکیده: اسانس‌های گیاهی از ترکیبات فرّاری تشکیل شده‌اند که غلظت‌های مختلف آن می‌تواند خاصیت سمّی و کشنده روی حشرات آفت داشته باشد. لذا در این پژوهش، اثرات کشنده و زیرکشنده ترکیبات ژرانیول و سیترونلال روی پارامترهای بیوشیمیایی و تولیدمثلی *Spodoptera frugiperda* مورد بررسی قرار گرفت. برای ترکیب ژرانیول مقدار LD_{30} ۹/۴۲میلی‌گرم بر گرم و LD_{50} ۱۳/۶۵ میلی‌گرم بر گرم استفاده شد، درحالی‌که برای سیترونلال از LD_{30} ۰/۰۶ میلی‌گرم بر گرم و LD_{50} ۰/۰۸ میلی‌گرم بر گرم استفاده شد. از استون خالص به‌عنوان شاهد استفاده شد. با استفاده از سرنگ ۵۰ میکرولیتری همیلتون مقدار ۱ میلی‌لیتر از هر غلظت روی پیش قفس سینه لارو سن سوم قرار داده شد. پس از ۴۸ ساعت، لاروها در محلول بافر سدیم فسفات با نسبت ۴ لارو در ۵ میلی‌لیتر بافر همگن‌سازی شدند. سپس مقدار پروتئین کل، قند کل، چربی و گلیکوژن تعیین شدند. هم‌چنین به‌طور روزانه طول دوره پیش از تخم‌گذاری، دوره تخم‌گذاری، دوره پس از تخم‌گذاری و تعداد تخم‌های گذاشته شده تعیین گردید. نتایج نشان داد که غلظت پروتئین کل و قند کل برای هر دو ترکیب در غلظت‌های مورد بررسی کاهش یافته است. هیچ تغییری در غلظت چربی مشاهده نشد. اما سیترونلال مقدار گلیکوژن را در هر دو غلظت افزایش داد. دوره تخم‌گذاری و تعداد تخم‌های گذاشته شده کاهش یافت. با این حال، دوره‌های قبل از تخم‌گذاری و بعد از تخم‌گذاری اختلافی نداشت. بنابراین نتیجه‌گیری می‌شود که ترکیبات ژرانیول و سیترونلال باعث تغییراتی در پارامترهای بیوشیمیایی می‌شوند که این امر در تولیدمثل حشرات کامل *S. frugiperda* مؤثر است.

واژگان کلیدی: لارو، ترکیبات استخراج شده، ترکیبات غذایی، تولیدمثل