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Phytochemical of *Piper* essential oil and acute toxicity against *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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ABSTRACT

Plant essential oils have been recognized as important natural resources for bioprospection of new insecticides. Here, we provided a chemical analysis and assessing the toxicity of the essential oil of *Piper aduncum* L. (Piperaceae) against *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) a cosmopolite and key pest in agricultural systems. The essential oil was obtained from the leaves of *P. aduncum* via hydrodistillation. Subsequently, acute mortality bioassays with two modes of exposure (ingestion and topical methods) were conducted to assess the lethal effects. The chromatograph analysis of *P. aduncum* essential oil indicated myristicin, isomyristicin, asaricim, dillapiole and isocroweacin the compounds more abundant. The acute toxicity bioassays exhibited concentration-dependent to mortality of larvae of first and third instar of *H. armigera*, when exposure by ingestion or topical application to *Piper* essential oil, with highlight to reduction in survival in 1st instar larvae. The median lethal time (LT_{50}) when larvae were treated by ingestion was of LT_{50} < 14.20 days (first instar) and LT_{50} = 16.89 days (third instar). Whereas to larvae treated by topical method the median lethal time was of LT_{50} < 14.68 days (first instar) and LT_{50} < 10.73 days (third instar), which demonstrate a drastic reduction in survival of insect, when contrasted to control. In summary, our results highlight toxicity of *P. aduncum* essential oil against *H. armigera* and provide more information on plant-compounds that can be use in bioprospection of new insecticide molecules to pest management.

Key words: chemical-control; pest-control; phytoinsecticide; Piperaceae; toxicology

Fitoquímica do óleo essencial de Piper e toxicidade aguda sobre Helicoverpa armigera (Lepidoptera: Noctuidae)

RESUMO

Óleos essenciais de plantas são reconhecidos como importantes recursos para bioprospecção de novos inseticidas. Aqui, provemos uma análise química e avaliamos a toxicidade do óleo essencial de *Piper aduncum* L. (Piperaceae) no controle *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) um inseto cosmopolita e praga-chave em sistemas agrícolas. O óleo essencial foi obtido a partir de folhas de *P. aduncum* via extrator do tipo Clevenger. Subsequente, foram conduzidos bioensaios de toxicidade aguda com dois modos de exposição (ingestão e tópico) para avaliar o efeito letal. A análise cromatográfica do óleo essencial de *P. aduncum* indicou myristicin, isomyristicin, asaricim, dillapiole e isocroweacin como os compostos mais abundantes. O bioensaio de toxicidade aguda indicou que a mortalidade foi dependente da concentração para larvas de primeiro e terceiro instar de *H. armigera*, quando expostas por ingestão e/ou aplicação tópica, com destaque para redução de sobrevivência para larvas de 1º instar. O tempo letal mediano (TL_{50}) para larvas expostas por ingestão foi de TL_{50} < 14.20 dias (primeiro instar) e TL_{50} = 16.89 dias (terceiro instar). Enquanto para larvas tratadas por aplicação tópica o TL_{50} < 14.68 dias (primeiro instar) e TL_{50} < 10.73 dias (terceiro instar), o que demonstra uma drástica redução na sobrevivência dos insetos, quando contrastado ao controle. Em resumo, nossos resultados evidenciam a toxicidade do óleo essencial de *P. aduncum* no controle de *H. armigera*, além disso, prove informações sobre compostos de plantas que podem ser usados na bioprospecção de novas moléculas inseticidas a serem incluídas no manejo de pragas.

Palavras-chave: controle-químico; controle-de-pragas; fitoinseticida; Piperaceae; toxicologia

Introduction

Insecticides are used to pest control, however many problems emerge when applied erroneous, such as pest resistance, population outbreaks and suppression of natural enemies, which make difficult of pest management (Fathipour & Sedaratian, 2013; Turchen et al., 2016). Alternatives strategies has been incorporated for lepidopteran control, like as genetically-modified plants (i.e. *Bacillus thuringiensis* toxins) (Chen et al., 2016), RNA interference (Qi et al., 2015), biological control agents (i.e., *Trichogramma* spp.) (Parra, 2014), pheromones baits (Zhang et al., 2012) and plant-compounds with activity insecticide (i.e., azadirachtin, pyrethrum, dillapiole, safrole, acetogenin, and others) (Lima et al., 2009; Mithofer & Boland, 2012; El-Wakeil, 2013).

Researches in bioprospection of plant-compound with proprieties repellents, deterrents (oviposition or feeding) or lethal to herbivorous insects (Mithofer & Boland, 2012) has emerged due needs incorporate new molecules and different modes of action (El-Wakeil, 2013) for chemical control management. In this context, plant essential oils have been recognized as important natural resources for new insecticides, with highlight to use in organic system.

Piper genus essential oil has exhibited promising results in pest control, because of active compounds, as terpenoid and phenylpropanoid that are broadly knowledge by toxicity on arthropods pest (Fazolin et al., 2007; Scott et al., 2007; Boulogne et al., 2012; Regnault-Roger et al., 2012), included to lepidopterans pest (Lima et al., 2009; Sanini et al., 2017). In addition, these compounds has been reported by exhibit low toxicity on egg parasitoids, which makes possible integrate with biological control (Turchen et al., 2016).

Remarkably, research in active plant-compound, as *Piper* spp. essential oil, are significant to provide information and about the potential in bioprospect of new active ingredients to pest management. Therefore, in this study, we provide chemical analysis of *Piper aduncum* L. (Piperaceae) essential oil and acute lethal effect of essential oil against *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), a cosmopolite insect and key pest in agricultural system, causing damage to crops.

Material and Methods

Insect populations

The populations of the *H. armigera* were collected by late 2013 in soybean fields in Tangará da Serra County (State of Mato Grosso, Brazil). Individual larva were reared in glass tubes and fed on artificial diet describe by Greene et al. (1976), with increment of vitamin solution. Pupae were removed and maintained in plastic pots (500 mL) up to adult emergence. Adults were transfer to PVC cages (diameter of 300mm) (one couple/cage), with paper inside used as substrate for egg laying and surface closed with *voil*. The insect adults are fed with honey-solution (30%) and beer (15%). They have been reared in controlled environmental conditions at 25 ± 2^{o}C temperature, 70 ± 10% relative humidity, and L12:D12 photophase, the same conditions in which the bioassays were carried out.

Plant material and extraction of volatile components

Plants of *P. aduncum* were collected from Tangará da Serra-MT (14°29'S and 57°54'W), in December 2013. These plants were identified in contrast with plants deposited by Piton et al. (2014) in the Tangará herbarium (TANG) with a voucher (registration number 1738). The preparation of the essential oil according with procedure used by Turchen et al. (2016), where summarized the leaves of *P. aduncum* were dried in a drying by 96h at 37°C, ground to a vegetable powder (1 mm diameter mesh), after the powder was subjected to hydrodistillation (100g by 3h), with an extractor type Clevenger. The hydrolat was partitioned with a separatory funnel using dichloromethane, and the obtained organic fractions were combined and dried over anhydrous calcium chloride. The salt was removed by vacuum filtration and the solvent evaporated at room temperature.

Chemical composition of the volatile material

The oil was analyzed by gas chromatography and mass spectrometry (GC-MS), with the following procedure used by Turchen et al. (2016), with followed sets: column, DB-5 fused silica (30 m x 0.25 mm i.d., 0.25μm film thickness), carrier gas helium (1.2 mL/min), temperature programed at 110–140ºC (5ºC min^{-1}), 140–290ºC (20ºC min^{-1}) and 290–330 (5ºC min^{-1}). Mass spectra, 70 eV (in El mode). The individual components were identified by comparison of both the mass spectra and their GC retention data with those authentic compounds previously analyzed and stored in the data system. Other identifications were made by comparing the mass spectra with those in the Wiley data system libraries (software class 5 k) and cited in the literature.

Bioassay design

Four concentrations (5, 10, 20 and 40 mg mL^{-1}) of *P. aduncum* essential oil and control (acetone P.A.) was used in the bioassay. For each concentration (plus the control) was prepared in 30 replicates in a completely randomized design. Acute mortality bioassays were performed with larvae of first (< 1 day old) and third instar and two modes of exposure (ingestion and topic).

In bioassay with exposure by ingestion, leaves of soybean were immersed in the concentration of essential oil by 3s and dried by 2h, after that leaves were offered as feed to larvae and each treatment contained 30 replicates (n = 30 larvae/treatment/instar). For topical exposure, larvae were treated by dorsal application with 1μL of treatment. After treatment, the insects were kept in plastic pots (145mL) with leaves of soybean (free essential oil) as feed for larvae. The insect survival, mortality, instar duration.

Statistical analysis

Normality and homoscedasticity of the data were checked using Shapiro-Wilk and Bartlett tests, respectively. The larvae survival exposed to essential oil through time were subjected to survival analysis using Weibull model estimators, and the survival curves were compared using contrast ($P < 0.05$). The median lethal time was estimated to all concentrations. The data of mortality (number of larvae death) were subjected to analyses of deviance and general linear model (GLM) with Poisson distribution and log as function link. Significant treatments were compared by contrasts ($P < 0.05$), always using the R software version. 3.1.1 (R-CoreTeam, 2014).

Results and Discussion

Phytochemical of essential oil

The chromatograph analysis of *P. aduncum* essential oil indicated that compounds more abundant were *myristicin* (24.00%), *isomyristicin* (20.99%), *asaricim* (20.00%), *dillapiole* (14.50%) and *isocroweacin* (18.00%), with the average yield of essential oil was 9.98 g kg^{-1} (Figure 1).

In our chemical analysis of *P. aduncum* essential oil show standard profile in the genus, as previously reported (Maia et al., 1999; Fazolin et al., 2007; Almeida et al., 2009; Potzernheim et al., 2012; Turchen et al., 2016). However, is important highlight that was observed a quantitative dissimilarity between compounds proportion, with emphasis to dillapiole (14.50%) that exhibited lower proportion in our study, when contrasted with other researches (Fazolin et al., 2007; Potzernheim et al., 2012).

The explanation for these results has relation with innumerous factors (e.g. region, seasonal, nutritional, and other), which can interfered in proportion of compounds in the plant (Almeida et al., 2009; Assis et al., 2013). This hypothesis can be reinforced with our results, since that we found similar chemical profile to previous research conducted in same region (Turchen et al., 2016).

The chemical analysis exhibited two promises compounds [myristicin and dillapiole]. The myristicin is widely distributed in plant kingdom and has been reported as insecticide against innumerous pest-insect and vector [i.e., *Spilarctia oblique* Walker (Lepidoptera: Arctiidae) and *Aedes aegypti* (L.) (Diptera: Culicidae)] (Srivastava et al., 2001). Similarly, the dillapiole that is broadly reported in *Piper* species (Scott et al., 2007) and have insecticide activity against in many insect orders (i.e., Coleoptera, Hymenoptera, Diptera, Hemiptera and Lepidoptera) (Fazolin et al., 2007; Piton et al., 2014; Turchen et al., 2016). However, innumerous research had suggested these compounds as synergic agent, since that can interfere on P450 activities in a wide range of organisms including insects (Schuler, 2011), which is very important to pest management, due improve effectiveness of pesticide.

Acute mortality bioassay

We detected significant reduction in survival time to larvae when exposure by ingestion (first instar: $\chi^2 = 17.45$, df. = 4, $P = 0.00157$, third instar: $\chi^2 = 10.808$, df. = 4, $P = 0.0288$). In larvae of first instar the lethal time was lower ($LT_{50} < 14.20 \pm 1.56$ days) in all essential oil concentrations, when contrasted with control ($LT_{50} = 21.32 \pm 1.56$ days) (Figure 2A). For larvae of third instar the concentrations of 5 - 20 mg mL^{-1}($LT_{50} = 20.20 \pm 1.27$ up to 18.82 ± 1.22 days) do not differ of control ($LT_{50} = 22.86 \pm 1.04$ days), but was observed lower survival in 40 mg mL^{-1}($LT_{50} = 16.89 \pm 1.14$ days) (Figure 2B).

Similar results were observed of larvae exposed by topical application with significant reduction in survival time (first instar: $\chi^2 = 39.929$, df. = 4, $P < 0.0001$, third instar: $\chi^2 = 17.614$, df. = 4, $P = 0.0014$). To larvae of first instar the median survival time in all essential oil concentrations was lower of 14 days ($LT_{50} < 14.68 \pm 1.86$ days) and differ of control ($LT_{50} = 19.35 \pm 1.89$ days) (Figure 3A). For larvae of third instar, with except to 5 mg mL^{-1} ($LT_{50} = 20.77 \pm 0.67$ days), all concentration differ of control ($LT_{50} = 24.12 \pm 1.05$ days) with survival lower that 10 days ($LT_{50} = 10.73 \pm 1.15$days) (Figure 3B).

Plant metabolites provide natural defense of plant to attack by arthropod herbivore, with highlight to secondary metabolites that are reported by act as repellents, deterrents (oviposition or feeding) or lethal to herbivorous (Mithofer & Boland, 2012). Curiously, a diversity of plants has been reported by cause toxic effect on *H. armigera*, like as mortality, deformation and prolongation of the larval stage (Baskar & Ignacimuthu 2012; Anshul et al., 2014; Paul & Moumita-Choudhury, 2016).

The results above-mentioned demonstrated that *Piper* essential oil had promising results for control of *H. armigera*, with significant impact on life cycle of insect. In our research, the curves showed a decrease in the survival rate of insect, which compromises directly the next generation, due drastic reduction in number of adults in the population. In addition, the essential oil was highly toxic for larvae, independent of modes of exposure, which could be clearly seen in survival curves (Figure 2, 3) and by lethal time parameters (Table 1).

Similar results on acute toxicity of *Piper* species on lepidopterans pest have been reported, for instance *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) – pest in maize (Murillo et al., 2014), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) – pest in tomato (Brito et al., 2015), *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae) – pest in

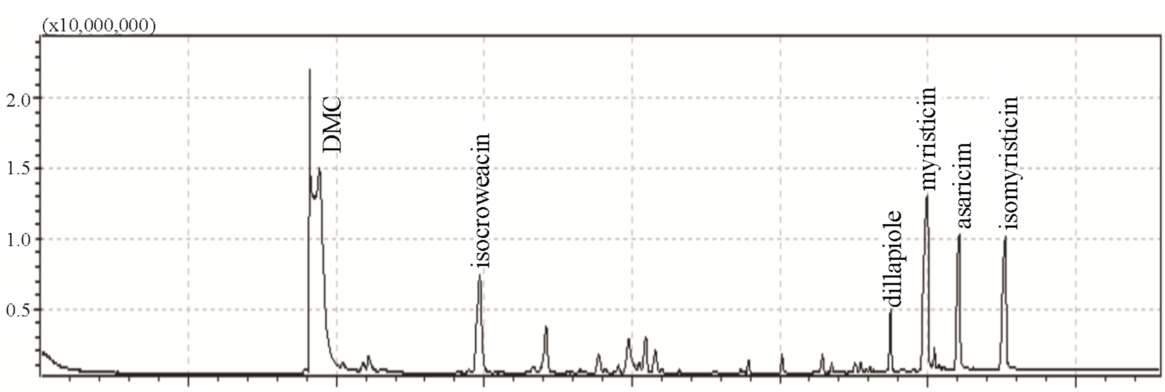

Figure 1. Chromatographic profile of chemical compounds in *Piper aduncum* L. (Piperaceae) essential oil.

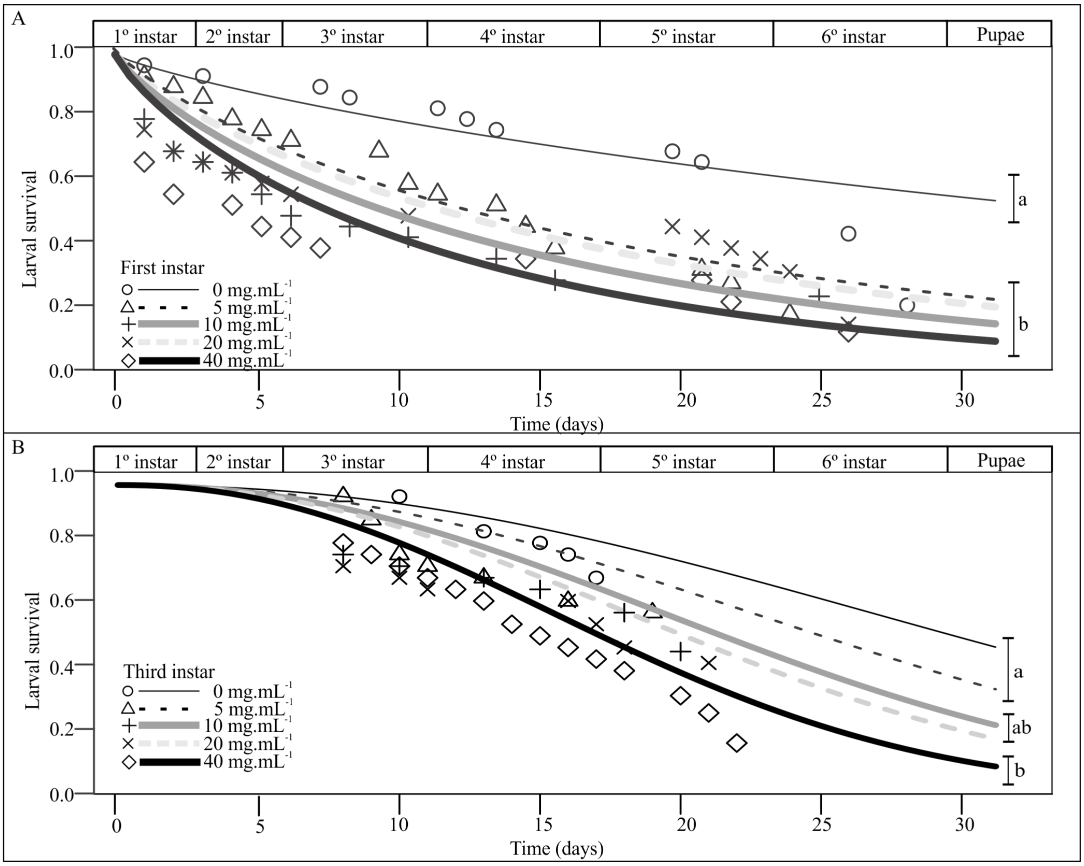

Figure 2. Larval survival of first (A) and third (B) instar of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), when exposed by ingestion to *Piper aduncum* L. (Piperaceae) essential oil.

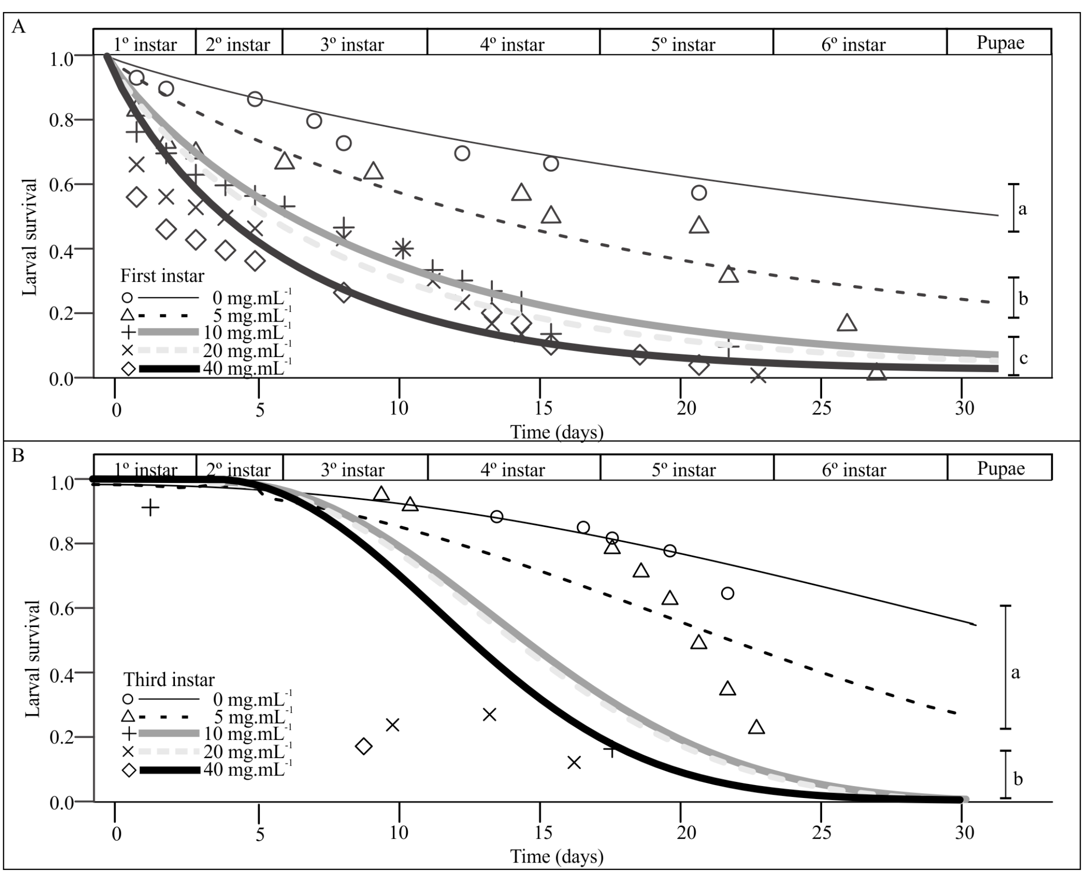

Figure 3. Larval survival of first (A) and third (B) instar of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), when exposed by topical application to *Piper aduncum* L. (Piperaceae) essential oil.

Table 1. Larval lethal time (LT_{50} - days) of *Helicoverpa armigera*, when first and third instar exposed by ingestion or topical application to *P. aduncum* essential oil.

Concentrations (mg mL^{-1})	Exposure by ingestion		Topical exposure	
	First instar[a]	Third instar[a]	First instar[a]	Third instar[a]
0.00	21.32±1.56 a	22.86±1.04 a	19.35±1.89 a	24.12±1.05 a
5.00	14.20±1.56 b	20.20±1.27 a	14.68±1.86 b	20.77±0.67 a
10.00	11.78±1.95 b	18.82±1.22 ab	8.68±1.30 c	10.60±1.10 b
20.00	13.61±2.05 b	19.56±1.52 ab	7.70±1.34 c	10.73±1.15 b
40.00	10.74±1.98 b	16.89±1.14 b	6.13±1.21 c	9.40±0.78 b

[a]Mean ± standard deviation (days) followed by the same letter in the column do not differ by multiple comparison for contrast test (P = 0.05).

sugarcane (Debonsi et al., 2008). In this perspective, our results indicate the potential of botanical insecticides in provide new molecules and likely new modes of action for pest management.

In contrast, problems in use of essential oil always are pointed in agriculture system, like as phyto-toxicity, fast degradation, low permeability, solubility, stability and, others. However, in last years, the application technology have improve to effectiveness of essential oil (i.e. nanoencapsulation), which improve solubility, permeability, and stability of compound, besides increasing their pest control efficacy for a longer period (Regnault-Roger et al., 2012). In addition, another advances on *Piper* essential oil refers to selectivity on natural enemies (i.e., eggs parasitoid - Platygastridae) (Turchen et al., 2016), which make possible association of essential oil to biological control. Thus, we believed that *Piper* essential oil provide promising compounds to bioprospection of molecules to be incorporated in pest-management.

Conclusions

We reveals phytochemical of *Piper aduncum* essential oil and provide more information on plant-compounds that can be use in bioprospection of new insecticide molecules to pest management. In addition, confirmed the toxicity of *Piper* essential oil against *Helicoverpa armigera*, a key pest in agricultural systems.

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