

ANTIFEEDANT AND REPELLENT ACTIVITIES OF *Melia azedarach* L. FRUIT EXTRACTS ON FALL ARMYWORM

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RESUMO:

O objetivo deste estudo foi avaliar a supressão do apetite e atividade inseticida de extratos das partes aéreas de *M.azedarach* sobre larvas de *S. frugiperda* (Lepidoptera: Noctuidae) obtido a partir da maceração por solvente, com uso de ultra-som e extração com CO₂ em estado supercrítico. Os extratos obtidos por diferentes métodos de extração (maceração, ultrassom e extração supercrítica), e diferentes solventes (etanol, n-hexano, acetato de etila e CO₂) foram testados sobre *S. frugiperda*, através da pulverização dos extratos sobre lagartas do terceiro instar alocadas em plântulas de milho. Atividade antialimentar e repelente foram observadas nos tratamentos com extratos de maceração com etanol dos frutos e ramos; extratos obtidos com solvente n-hexano e CO₂ supercríticos dos frutos, os quais reduziram significativamente o dano foliar nas plântulas, com equivalência ao controle positivo (inseticida comercial). Em relação à mortalidade das larvas, o extrato CO₂ supercrítico dos frutos foi o que apresentou maior mortalidade entre os extratos testados, igualando-se ao tratamento com inseticida comercial, o que presume que este extrato é o mais promissor para o controle do inseto em culturas de milho.

Palavras-chave: cinamomo, extração por ultrassom, extração CO₂ supercrítico, *Spodoptera frugiperda*.

ABSTRACT:

The aim of this study was to evaluate the appetite suppression and insecticidal activity of *Melia azedarach* aerial component extracts on the larvae of *Spodoptera frugiperda* obtained from the maceration by solvent, ultrasound and supercritical CO₂ extraction. Extracts of aerial components of *M. azedarach* were obtained by three different extraction methods (maceration, ultrasound and supercritical extraction) using different solvents (ethanol, n-hexane, ethyl acetate and CO₂) for use in testing appetite suppression activity in *S. frugiperda* (Lepidoptera: Noctuidae). Extracts were sprayed on third instar larvae, which were then placed on corn seedlings. Extracts prepared by ethanol maceration of the fruits and branches showed appetite suppression and repellent activity, extracts of fruits obtained with n-hexane solvent and supercritical CO₂ resulted in significantly reduced leaf damage in the corn, with a result equivalent to the positive control (commercial insecticide). Regarding larvae mortality, the supercritical CO₂ extract of fruits resulted in the highest *S. frugiperda* mortality among the tested extracts, equivalent to treatment with positive control. This extract appears to be the most promising for controlling this corn crop insect pest.

Keywords: chinaberry, ultrasound extraction, supercritical CO₂ extraction, *Spodoptera frugiperda*.

Large-scale food production demands the use of large quantities of pesticides, which have a high bioaccumulation factor in many organisms. The growth rate in production and use of pesticides exceeds the knowledge of their actual effects on individuals and the environment, particularly with regard to synergistic toxic effects (Pedlowski et al. 2012). The application of plant extracts for control of agricultural insect pests has been identified as a management tool with less environmental impact, because the extracts are

biodegradable and produce significantly less waste (Ntalli & Menkissoglu-Spiroudi 2011).

A wide variety of plant families have species that exhibit potent insecticidal compounds. The Meliaceae family has been identified as one of the most promising groups, since most of their species have several isolated compounds with demonstrated insecticidal activity (Castillo-Sánchez et al., 2010). Among the Meliaceae species, *Melia azedarach* L. is chemically characterized by the presence of compounds having antifungal,

insecticidal (Carpinella et al. 2006, Cabralet al. 2008), nematocidal (Cavoski et al. 2012), acaricidal (Sousa et al. 2011) and larvicidal activity (Al-Mehmadi & Al-Khalaf 2010), among others.

The fall armyworm *Spodoptera frugiperda* (JE Smith, 1797) (Lepidoptera: Noctuidae) is considered one of the most destructive insect agricultural pests, occurring throughout the year in tropical regions (Murua & Virla 2004). It is the main insect pest of corn because it feeds on almost all stages of plant development, with preference for cartridge younger plants (Cruz & Turpin 1982).

Studies have reported effects of *M. azedarach* extracts on different insect pests (Prophiro et al. 2008, Dequech et al. 2000, Kebede et al. 2010, Defagó et al. 2011, Bullangpoti et al. 2011), obtained by use of polar solvents such as ethanol, methanol and water. As the solvent type and extraction method used will directly impact the composition of the extract and consequently its activity, then is important evaluate the activity resulting from each extraction methodology. Accordingly, the aim of this study was to evaluate the appetite suppression and insecticidal activity of *M. azedarach* aerial component extracts on the larvae of *S. frugiperda* obtained from the maceration by solvent, ultrasound and supercritical CO₂ extraction.

MATERIAL AND METHODS

Plant material and extraction methods.

Samples of *M. azedarach* aerial components were collected in Chapecó, Santa Catarina, Brazil, located at 27°05'38.08" south and 52°40'00.52" west in February 2013 (summer). The plant material was dried at 40 °C for 2 days, until water content was less than 0.5-wt% (determined using Karl Fisher titration); the dried material was then milled in an industrial blender, size up to 2 mm.

Type specimens were deposited in the Universidade Comunitária de Chapecó (Unochapecó, SC, Brazil) herbarium under the accession number UNO 2841.

The experimental supercritical CO₂ extraction apparatus were performed in a laboratory scale unit, which consists of a CO₂ reservoir, a thermostatic bath, a syringe pump (ISCO 260D), a 0.518 L jacketed extraction vessel, and an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201) with ± 0.12 bar precision. 70.03 $\pm$ 0.09 g of dried and powdered fruit were charged into the extraction vessel. The experiments were performed isothermally, at 60 °C and constant pressure, 250 bar, using a mass CO₂ flow rate of 2 g min⁻¹ over 2 h. These conditions were selected based on the maximum extraction yield obtained by Scapinello et al. (2014).

A fractionation step was then performed on the supercritical extract using a liquid-liquid extraction technique. 50 mL of water was added to 10 g of extract, after which n-hexane solvent was used to remove the organic phase, resulted of the hexane fraction of the supercritical extract. In the aqueous phase, ethyl acetate was added to obtain the ethyl acetate fraction of the supercritical extract. The solvents were then removed via evaporation.

The maceration was realized with the dried and ground *M. azedarach* fruits with 99% ethyl alcohol in a ratio of 1:3 grams of plant material per liter of solvent, and homogenized for three days at 24 hours intervals. Thereafter, the extract was filtered and the solvent removed in rotary evaporator under reduced pressure. The same procedure was performed for branches and leaves.

For ultrasound extraction, the dried and ground fruits and with 99% ethyl alcohol in a ratio of 1:3 were placed in a 250 ml capacity Erlenmeyer flask equipped with a lid, where the mixture underwent an extraction

ultrasound bath (Ultrasonic® model USC 2800A, capacity 9.5L frequency of 40 KHz) individually for 2 hours at $40 \pm 1^\circ\text{C}$. The extract was then filtered and concentrated in a rotary evaporator under reduced pressure. The mass of fruit used in the extraction was 50.04 ± 0.026 g. The same procedure was performed for the ethyl acetate and n-hexane solvents.

The phytochemical screening of the extracts was accomplished as described by Harbore (1998) by observing the colorimetric variation after the addition of specific reagents on silica gel plates 60 F254(Merck®). The main phytochemicals analyzed were terpenes, sterols, flavonoids, alkaloids, coumarins and tannins.

Antifeedant and toxicity assay.

The experimental design was completely randomized. Each treatment consisted of four corn plants from the open-pollinated Catarina variety, with heights of 12 - 15 cm. One *S. frugiperda* third instar larva (raised in the laboratory on artificial diet) was placed in each plant container. The plant containers were placed in trays with water to reduce the probability of caterpillars escaping the plants.

A 10% *M. azedarach* extract was prepared by mixing 6.40 g of extract in 64 ml of 0.5% Tween 80 aqueous solution, for the ethanolic maceration extracts of the fruits, branches and leaves. To prepare the 5% solution, we used 3.2 g of extract in 64 ml of 0.5% aqueous solution, for the supercritical extract, fractions of supercritical extract and ultrasound extracts of fruits. The insecticide Lannate® BR, with the active ingredient Methomyl, was used as a positive control in a dose equivalent to 0.6 L/ha (Brasil 2012). The negative control consisted of a 0.5% aqueous Tween 80 solution.

Experimental observations were made both one day and two days after commencing the experiment, at which time we registered the

number of dead caterpillars, the number of missing caterpillars and damage extent of the seedlings. Foliar damage values were assigned as follows: consumption above 3 cm² was assigned a value of 5; between 2 and 3 cm² was assigned a value of 4; between 1 to 2 cm² was assigned value 3; between 0.5 to 1 cm² value 2; between 0.5 to 0 cm² value 1, and no consumption was assigned a value of 0.

Statistical analysis.

The results of extraction yield and biological experiments are in fact expressed as mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) followed by Tukey post hoc test to identify significant differences between groups. Differences were considered to be significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Among all solvent types employed for *M. azedarach* extraction, ethanol extraction produces a significantly higher yield than other extraction solvents (Table 1). This is likely due to the difference in polarity among solvents; ethanol is a solvent of medium polarity, and thus contains a greater quantity of solubilizing compounds, both polar and nonpolar. In a supercritical extraction using a nonpolar solvent, the compounds with high polarity are not well dissolved in the solvent, and the extraction efficiency is lower.

The ultrasound extraction yield with n-hexane was not significantly different from the extraction with supercritical CO₂, probably because of the similar polarity of these two solvents. However, the CO₂ supercritical extraction has an advantage over the extraction with n-hexane, as this process does not require solvent removal after extraction (and the obtained extract is completely free of solvent), which makes it safer for in vivo biological tests. This fact generally is reported how one of the main reasons for the use of this extraction technique (Silva et al., 2016).

Table 1. Yield of different extraction methods of *M. azedarach* fruits.

Extraction method	Temperature (°C)	Time (h)	Yield (%)
Maceration			
Ethanol	27	72	10.37±1.25a
Ultrasound			
Ethanol	40	2	9.68±0.30a
Ethyl acetate	40	2	3.18±0.25b
n-Hexane	40	2	2.81±1.29b
Supercritical			
CO ₂	40	2	2.65±0.16b

Values followed by the same letter in columns do not differ by Tukey's test at a 95% confidence level.

The ultrasound extraction proved to be a faster and more efficient method than conventional maceration to extraction of natural product from plants, obtaining an equivalent yield in less time. Similar results was obtained by Porto et al. (2013) for extraction of oil from grape seeds, which reported the advantage of the ultrasound, compared to the conventional extraction methods, besides the comparable oil yield, the active compounds yield (polyphenols) was also similar, with a lower solvent consumption and a shorter extraction time.

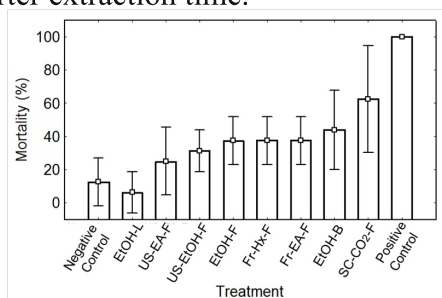


Figure 1. *S. frugiperda* caterpillar mortality on corn seedlings treated with *M. azedarach* extracts. □ mean; I standard deviation. EtOH-F: Maceration with ethanol of the leaves at 10%; US-EA-F: Ultrasound extraction with ethyl acetate of the fruits at 5%; US-EtOH-F: Ultrasound extraction with ethanol of the fruits at 5%; EtOH-F: Maceration with ethanol of the fruits at 10%; Fr-Hx-F: N-hexane fraction of SC-CO₂ extract of the fruits at 5%; Fr-EA-F: Ethyl acetate fraction of SC-CO₂ extract of the fruits at 5%; EtOH-B: Maceration with ethanol of the branches at 10%; SC-CO₂-F: supercritical CO₂ extraction of the fruits at 5%.

Phytochemical analysis of *M. azedarach* fruit extracts obtained by CO₂ supercritical extraction, ultrasound extraction with n-hexane, ethanol and ethyl acetate solvents, and by ethanolic maceration all demonstrated the presence of terpene compounds. Terpenes present in species of Meliaceae family are associated with appetite suppression and/or growth inhibition in insects (Araújo et al. 2009). Compounds in the coumarin class, and sterols were also found in these extracts. Just as with the fruits, the branch extracts indicated presence of terpenes, sterols and coumarin compounds. Compounds in the tannin and flavonoid classes were found only in the leaf ethanol extracts. The presence and concentration of secondary metabolites in plant extracts was not only related to the extraction method, but also to the part of the plant used, and environmental factors related to plant location (soil composition, altitude, climate, rainfall, among others), according Figueiredo et al. (2008).

The use of extracts obtained from different *M. azedarach* structures applied to corn plants mainly resulted in feeding suppression of insects, with significantly reduced damage to corn seedlings (Table 2). A repellent effect also occurred, as many larvae were found in the water trays below the plant containers, indicating that insects sought to evade the plant instead of remaining housed in the seedling containers.

The assessment of leaf consumption was directly linked to the appetite suppressing action of the extracts on the fall armyworm. For the ten extracts tested, low leaf consumption was observed in the use of SC-CO₂ fruit extracts, ethanol branch and fruit extracts, and n-hexane fraction of the supercritical extract of fruits. Leaf consumption after application of these extracts was not significantly different than the positive control, which indicates that they were as efficient as the commercial insecticide

Lannate® in controlling insect damage to the seedlings.

Among the extracts tested, supercritical extract of *M. azedarach* fruit proved to be the most promising for the control of *S. frugiperda*, as it caused the death of 62.5% of the insects and resulted in the lowest average foliar consumption value. This result was not significantly different than the positive control (Table 2), indicating insecticidal action similar to commercial insecticide. According Ntalli et al. (2014) work, the *Melia azedarach* is a plant species whose fruits possess nematicidal and insecticidal activity. In their studies, the fruit extracts disturbed development of *S. exigua*.

Table 2. Foliar consumption and mortality of *S. frugiperda* on corn seedlings sprayed with *M. azedarach* extract

Treatment	Foliar consumption	Mortality (%)
<i>Experiment 1</i>		
Negative control	3.31 ±0.38a	12.50a
EtOH Leaves 10%	2.66 ±0.52ab	6.25a
EtOH Fruits 10%	1.66 ±0.43bc	37.50a
EtOH Branches 10%	1.31 ±0.72c	43.75a
Positive control	1.50 ±0.20c	100.0b
<i>Experiment 2</i>		
Negative control	3.19 ±0.80a	12.50a
SC-CO ₂ Fruits 5%	1.13 ±0.25b	62.50bc
Fr SC-AB Fruits 5%	3.75 ±0.93ab	37.50a
Fr SC-Hx Fruits 5%	2.56 ±0.72b	37.50a
Positive control	0.44 ±0.13b	100.0c
<i>Experiment 3</i>		
Negative control	4.50 ±0.54a	12.50a
US-EtOH Fruits 5%	3.56 ±0.24ab	31.25a
US-AB Fruits 5%	3.19 ±0.69ab	25.00a
US-Hx Fruits 5%	3.19 ±0.13ab	50.00a
Positive control	0.88 ±0.52c	100.0c

The electron microscopic studies revealed the extracts caused malformations of fat body and midgut cells, and the most common observed malformations were: vacuolization of mitochondria, swollen endoplasmic reticulum and degeneration of nuclei.

When we evaluated the insecticidal effect of different plant sections, we found that *M. azedarach* fruit extract application resulted in the least damage to corn leaves, followed by extracts from branches. Leaf extracts had no adverse effects on the fall armyworm, with results similar to the negative control. Extracts obtained with nonpolar solvents resulted in increased larval mortality, indicating that oily extracts are more effective in controlling this species (Figure 1). Extracts obtained using polar solvents may contain high sugar concentration, and therefore may be less efficient in controlling insects. Céspedes et al. (2013) used *Condalia microphylla* Cav extracts (Rhamnaceae) in the control of *S. frugiperda* larvae, and the extract obtained with n-hexane had higher antifeedant effect than the other extracts tested (ethyl acetate, methanol and water). According to these authors, when the aqueous extract was used, the effect was the opposite and the caterpillars preferred the treated leaves rather than control leaves.

These results demonstrate the importance of evaluating plant bioactivity resulting from different extraction methods and solvents. One cannot assert that a plant is not bioactive simply because particular plant parts do not have bioactive effects, when, for example, water is used for obtaining the extract. It might be that the extract of the same plant structure can present bioactive effects when prepared from nonpolar solvents, such as n-hexane and supercritical carbon dioxide. The extraction yield of the active component of the plant will also depend on its solubility in the solvent used.

CONCLUSION

The extracts obtained from ethanol maceration of *M. azedarach* fruits and branches, extracts obtained with n-hexane and SC-CO₂ of the fruit had a feeding suppression and repellent effect on the Fall armyworm,

seedlings. For the SC-CO₂ extract of the fruits was observed insecticidal action equivalent to the commercial product proving to be a method of extraction efficient and environmentally friendly for being a clean technology.

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