EFFECTS OF SFE *Melia azedarach* ON THE CONTROL OF FALL ARMYWORM (*Spodoptera frugiperda*)

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Abstract. To reduce the use of pesticides in agriculture, new methods of insect control are under investigation towards lower environmental impact. The aim of this work was to evaluate the insecticidal potential of extracts of milled fruits of *Melia azedarach* obtained by supercritical carbon dioxide extraction (SC-CO_2) for the control of fall armyworm (*Spodoptera frugiperda*). For this purpose, the effects of temperature, pressure, static time, extraction time and particle size were investigated. In the test of biological activity four artificial diets were prepared with different SC-CO_2 extract concentrations (100, 500, 1000 and 5000mg/Kg) and offered to *S. frugiperda* (Lepidoptera, Noctuidae), evaluating mass and body size of insects during larval and pupal stages, and mortality during all phases of the insect biology. The higher extraction yield was obtained at 60°C and 250bar, static time 60min and particle size of 0.85mm. Secondary metabolites from the class of coumarins, sterols and terpenes were identified in the extract, and the triterpene melianone a major constituent. In biological tests, during the larval stage of the insect, mortality increased with increasing extract concentration in the diet, with 50% mortality (LC_{50}) at a concentration of 376.74mg/Kg and reaching 100% at the concentration of 5000mg/Kg. At concentrations of 500, 1000 and 5000mg/Kg, the extract showed anti-feedant activity, resulting in larvae and pupae with mass and body length significantly smaller than control. At the concentration of 100mg/Kg it was observed bioactive effects from the adult stage of the insect, presenting morphological deformities, hence indicating chronic toxicity effect.

Keywords: Supercritical extraction, *Spodoptera frugiperda*, chinaberry, bioinsecticide.

1. Introduction

Synthetic pesticides possess quick action, but they are often toxic to mammals and non target organisms, and have low degradation rate, remaining in the environment for a long time, hence contaminating the air, soil and water [1]. The speed of growth in the production and use of such products is much larger than the real knowledge about effects on both individuals and environment, and further unknown in relation to the synergistic toxicity [2].

The need of developing ecological pesticides is urgent, due to the increasing pesticide safety requirements, and alternative most used to this is obtain extracts from plants that have chemically active compounds.

Pesticides of biological origin are often as effective as the synthetic pesticides, non-toxic for most species of warm blood, reach only the target species and are easily degraded in the presence of ambient air and light [3-4].

Insecticides plant extracts have been traditionally obtained by means of organic solvents, which must be removed after the extraction process, generating large quantities of wastes and emissions of these compounds to atmosphere. The supercritical fluid extraction (SFE) is a promising technology to overcome these disadvantages [5]. The solvent most widely used is carbon dioxide, due to its easy recovery by simple decompression and provides free solvent analytes. Moreover, for the extraction of natural products, the technique requires lower temperatures and provides a non-oxidizing mean, which allows the extraction of thermolabile compounds or that are easily oxidized [6-8].

Many insecticide substances derived from plants have high solubility in carbon dioxide under supercritical conditions, with considerable yields of these compounds from SFE [4]. Compounds of terpenes class, which have recognized insecticidal potential, have already been extracted of meliaceous *Azadirachta indica* A. Juss (neem) through SFE by Ambrosino et al. [9], Tonthubthimthong et al. [10] and Ismadji et al. [11]. Another species belonging to Meliaceae family, less studied than the aforementioned, is the *Melia azedarach*, which also has proven insecticidal properties in their extracts obtained by conventional extraction methods [12-17], but little studied in SFE.

In this work the insect antifeedant effects of SFE of *M. azedarach* were tested against *Spodoptera frugiperda* (fall armyworm), an insect of the order Lepidoptera [18], well known for its voracity during the larval stage, when it feeds mainly on leaves, buds, flowers and fruits of various species of crop plants such as rice, maize, sorghum and some cruciferous [19-21], have their occurrence in the Americas, with confirmed activity throughout the year in tropical regions.

2. Materials and method

2.1 Plant material

Samples of fruits of *Melia azedarach* were collected during summer 2012 (February), when its color was light green and smooth epidermis, in the city of Chapecó located on South Brazil (27°05'38" S, 52°40'0.5" W). Voucher specimens were deposited on the Herbarium of Community University of Chapecó Region (Herbarium Unochapecó) with the accession number of UNO 2841. Plant material (fruit) was dried (40°C for two days) and then milled in industrial blender. The samples were then stored at room temperature under nitrogen atmosphere prior to the extraction.

2.2 Supercritical CO_2 extraction

Carbon dioxide (99.9% purity in the liquid phase) was kindly provided by White Martins S.A. The experimental extraction apparatus and procedure have been described in detail in a variety of studies [22-25]. Briefly, the experiments were performed in a laboratory scale unit, which consists basically of a CO_2 reservoir, two thermostatic baths, a syringe pump (ISCO 260D), a 0.518L jacketed extraction vessel, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201) with a precision of ± 0.12 bar, a collector vessel with a glass tube and a cold trap. Amounts of around 70.03g ± 0.09g of dried and powdered fruits were charged into the extraction vessel. The CO_2 was pumped into the bed, which was held by two 300 mesh wire disks at both ends, and was kept in contact with the sample matrix for at least one hour to allow the system stabilization at the same condition of the experiment. Afterwards, the essential oil was collected opening the micrometering valve and the CO_2 mass flow was accounted for the pump recordings.

Effects of temperature and pressure. To investigate the effect of extraction temperature and pressure on the yield (w/w %) of fruit extraction of *M. azedarach*, it was employed a full 2^2 factorial experimental design with triplicate at the center point, considering as main variables extraction temperature in the range of 313K to 333K and pressure from 150 bar to 250 bar, as recommended by Yu et al. [26] for the extraction of limonoids of plant tissues. In these tests, the static time was fixed at 120 min, the extraction time at 90 min, the mass CO_2 flow rate of 2g/min and fruit size less than 4.0 mm.

The extraction yield is defined here as the weight percentage of the oil extracted with respect to the initial charge of the raw material in the extractor.

Effects of static time. Extractions with different contact times of supercritical CO_2 with the fruits of *M. azedarach* were performed, evaluating the yield of extraction (w/w %) at 60, 120 and 180 minutes, keeping constant the extraction time of 90 minutes and temperature and pressure mentioned before.

Effects of particle size of fruits *M. azedarach*. To evaluate the effect of particle size on the extraction yield, extractions of fruits were performed with three different particle sizes: 0.85, 2.0 and 4.0 mm, with data collection at 15, 30, 60, 90 and 120 minutes of extraction, obtained from a series of Tyler sieves, mesh 20, 10 and 5, respectively. The fixed parameters in this evaluation were contact time cited before, extraction time of 90 minutes, and temperature and pressure established aforementioned.

2.3 Phytochemical analysis

The phytochemical screening was accomplished as described by Harbore [27], observing colorimetric variation after the addition of specific reagents, in silica gel plates 60 F254 (Merck®). The main phytochemicals analyzed were terpenes, sterols, flavonoids, alkaloids, coumarins and tannins.

ESI-Q-TOFMS measurements were performed with a micrOTOF Q-II (Bruker Daltonics) mass spectrometer equipped with an automatic syringe pump from KD Scientific for sample injection. The ESI-QTOF mass spectrometer was running at 4.5 kV and a desolvation temperature of 180 ºC. The mass spectrometer was operating in the positive ion mode. The standard electrospray ion (ESI) source was used to generate the ions. Sample was injected using a constant flow (3µL /min). The solvent was an acetonitrile/methanol mixture. The ESI-Q-TOF MS instrument was calibrated in the range m/z 50–3000 using an internal calibration standard (Low concentration tuning mix solution) which is supplied from Agilent Technologies. Data were processed via Bruker Data Analysis software version 4.0.

2.4 Bioassay test against *Spodoptera frugiperda*

The extract used in the biological tests was obtained by extraction SC-CO2 at 250bar and 60°C from the fruits of M. azedarach. The bioassay was conducted with four concentrations of SC-CO2 extract (100, 500, 1000 and 5000 mg of extract by Kg of diet) offered to the larvae of S. frugiperda J.E. Smith (Lepidoptera: Noctuidae) with seven days old, which had an average length of 0.65 cm (±0.05 cm) and a weight of 2.72 mg (±0.37 mg). The larvae were kept individually in capped plastic containers (80 mL) together with the diet, at a temperature of 25.0°C (±0.5 °C). The experimental design was executed completely random. In each treatment it was used 10 larvae, in four replicates. The artificial diet used in the experiment was prepared as described by Greene et al. [28].

The evaluations were performed twice a week, recording the number of dead individuals in each treatment, the average length and weight of each caterpillar survivor since the implementation of the experiment until adulthood insect, resulting in 40 days of the experiment. Mortality larvae data were corrected for control mortality using Abbott's formula [29] and the LC_{50} (concentration that produces 50% mortality) was determined by logPROBIT analysis.

3. Results and discussion

3.1 Supercritical CO_2 extraction

Effects of temperature and pressure. Through the analysis of variance, it was found that only the pressure variable had significant influence on the extraction yield of the fruits of *M. azedarach* with supercritical CO_2 (confidence level of 95%). Extraction temperature and the cross effect of temperature and pressure showed no significant influences. It was possible to observe higher yields in assays with higher pressures (Table 1). This result was also reported by other authors in their studies with SC-CO_2 extraction from different plants [30-32].

The efficiency of extraction depends on the solubility of the compounds in the supercritical solvent used. The solvating power of supercritical CO_2 is a direct consequence of its density and can be represented by a nonlinear function of pressure and temperature operation [33].

From Table 1 it is observed that increasing the system pressure at isotherm conditions, results in a raise in solvent density, and accordingly an increase in solvent power. On the other hand, if system temperature is increased at isobaric conditions, there is a reduction in solvent density, but an increase in the vapor pressure of the solute.

Table 1. Experimental data and the observed response values with different combinations of temperature (T) and pressure (P) for *M. azedarach* fruits by SFE

Coded parameters		Actual parameter values		CO_2 density[(1)] (g/L)	Yield (%, w/w)
T	P	T (°C)	P (bar)		
-1	-1	40	150	781.6	2.32 ±0.45
+1	-1	60	150	607.2	0.48 ±0.10
0	0	50	200	785.0	4.10 ±0.50
-1	+1	40	250	880.2	3.98 ±0.18
+1	+1	60	250	787.2	5.15 ±0.35

[(1)] SPAN; WAGNER, 1996.

The response surface of Fig. 1 shows that at 250 bar the extraction yield was higher at 60°C, since at a pressure of 150 bar, the highest yield was obtained at 40°C, occurring then, a crossover in the extraction yield curves. This phenomenon is known as retrograde condensation [34-35], which is a result of the predominance of only one of the factors that influence the solubility of solute in CO_2 (solute vapor pressure or solvent density).

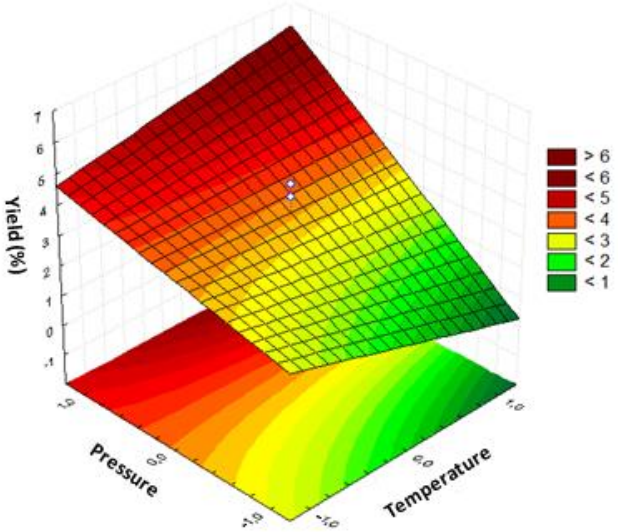

Figure 1. Graph of response surface for the yield of extraction SC-CO_2 of fruits of *M. azedarach* in relation to pressure and temperature

At higher pressure level (250bar) the effect of vapor pressure predominates and the higher the system temperature, the greater solute vapor pressure, implying in the increase extraction yield. However, at lower pressures (150 bar) the behavior is opposite, due to the predominance of solvent density (solvent power), and an increase in temperature will cause a reduction in solvent density and thus a reduction in the extraction yield. Thus, it can be inferred that the effect of temperature on SFE is more complex, because affecting both the density of the solvent as the molecular mobility in general [32, 36-38]. Similar results were also found for SC-CO_2 extraction of seeds of *Rosa canina* L. (Rosaceae), and extraction of *Jatropha curcas* L. (Euphorbiaceae), with crossover curves by pressure for different isotherms [39].

Effects of static time. The time that the fruits remained in contact with supercritical CO_2 before the extraction itself (solvent flow through vegetable bed) resulted in an increase in the extraction yield with the increase of static time, however, this increase was not significant (Table 2). This behavior is related to the solubility of the solute in the supercritical fluid. Usually, larger contact times between the solid matrix and the solvent promotes a greater solubilization, but when the solubility value of the solute in a given solvent is reached, greater contact time will not result in an increase in solubility, which was also observed in other studies with SC-CO_2 extraction of other plants [30, 40-41]. The time of equilibrium (saturation) depends

largely on temperature, but generally a static time of up to 30 minutes is reported by some authors as sufficient for extraction of natural products with supercritical CO_2 [42-43].

Table 2. Extraction yield in relation to the static time

Time (min)	Yield (%)
60	4.32 ±0.49 [a]
120	4.45 ±0.53 [a]
180	4.65 ±0.39 [a]

Values followed by the same letter in columns do not differ byTukey test at significance level of 95%.

Effects of particle size of fruits *M. azedarach* and extraction kinetics. The decrease of particle size of fruits *M. azedarach* resulted in increased yield of SC-CO_2 extraction (Fig. 2). The improvements in extraction yield might be explained in terms of the positive effect of reduced particle size on the internal resistance to mass transfer in solid matrix, which is relevant in process SFE. Thus, the rate of extraction is increased due to shortening of diffusion path [44]. Additionally, the milling process produces smaller particles, which causes an increase in specific surface area (surface area-to-volume ratio), and rupture of cell walls and other internal barriers to mass transfer, leaving the solute more accessible to supercritical solvent [45-46].

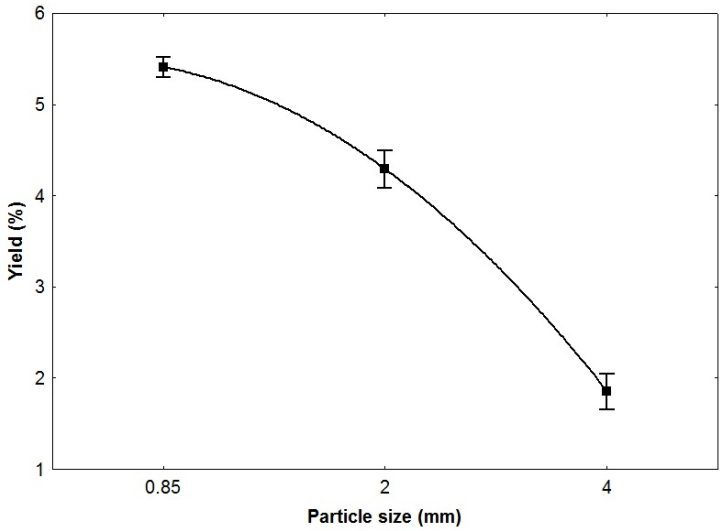

Figure 2. Extraction yield SC-CO_2 in relation to the particle size of fruits of *M. azedarach*. ■ mean, **I** mean ± standard deviation

When evaluating the extraction yield with respect to time (extraction kinetics), the effect of particle size on the mass transfer of SC-CO_2 extraction (Fig. 3) can be better visualized. For small fruit sizes, the extraction yield increased significantly until 120 min, and for larger fruit sizes, the yield reached an asymptotic behavior after 60 min.

Is can be observed from Fig. 3 that in the initial extraction phase the yields obtained with different fruit sizes were similar, which is explained by initial period of extraction kinetics, named as constant extraction rate (CER), where the solvent removes easily the more accessible solutes, which are on the particles surface. A higher CER can be noted for the fruits with size of 0.85 mm, just because the duration of this period is directly proportional to the amount of disrupted cells [47].

As the extraction takes place, the solutes more accessible will be depleted, and from that moment, the internal resistance of mass transfer will become limiting fact for the extraction rate (FER and DP periods) [48]. Thus, the particle size has a direct influence on the yield, since smaller particles favor the internal diffusion process, due to the shortest distance of solvent to intraparticle solute molecules. In the extraction kinetics it was verified that the CER period represents over 80% of extraction yield, and according to Meireles [49], although more accurate mathematical models are generally required for the entire process

simulation, a simple mathematical model that describes the CER period can be sufficient for a preliminary analysis of the feasibility of SFE.

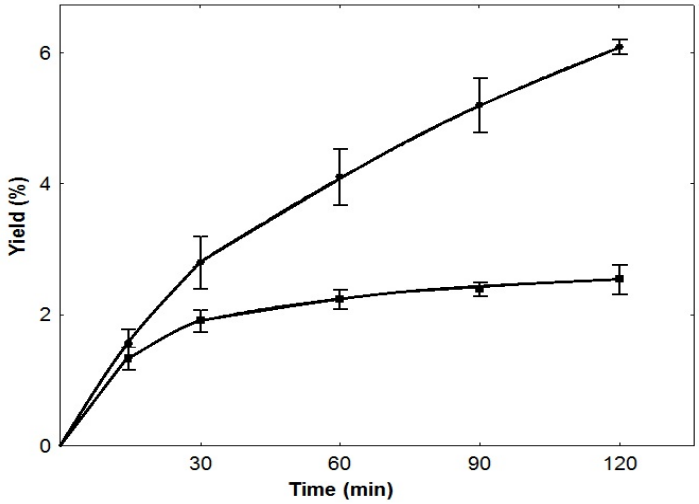

Figure 3. Kinetics of SC-CO_2 extraction for two sizes of fruits of *M. azedarach*. -●- mean fruit diameter 0.85mm; -■- mean fruit diameter 2mm, I mean ± standard deviation

Results obtained in this work are in complete agreement with other studies available in the literature [50-56], which show that the diffusion of the solvent inside the particle and solvent with solute outside the vegetable particles is the period that can limit the SC-CO_2 extraction process, and then the solid preparation (milling) is a key step for the design and feasibility of the extraction process.

3.2 Phytochemical analysis

In the SC-CO_2 extract of fruits of *M. azedarach* it was confirmed the presence of terpenes compounds, sterols and coumarins, with higher amounts of compounds from the class of terpenes, which are associated with the insecticides effects on the insects. Through analysis of ESI-Q-TOFMS it was observed the presence of the compound melianona in the extract (Fig. 4), a triterpene as the major constituent of *M. azedarach* [57]. In spectral measurements this compound exhibited a $[M + Na]^+$ at m/z 493.3 for $C_{30}H_{46}O_4$. This compound has antifeedant activity against larvae *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae) [58] and on *Reticulitermes speratus* (Kolbe) (Isoptera: Rhinotermitidae) [59].

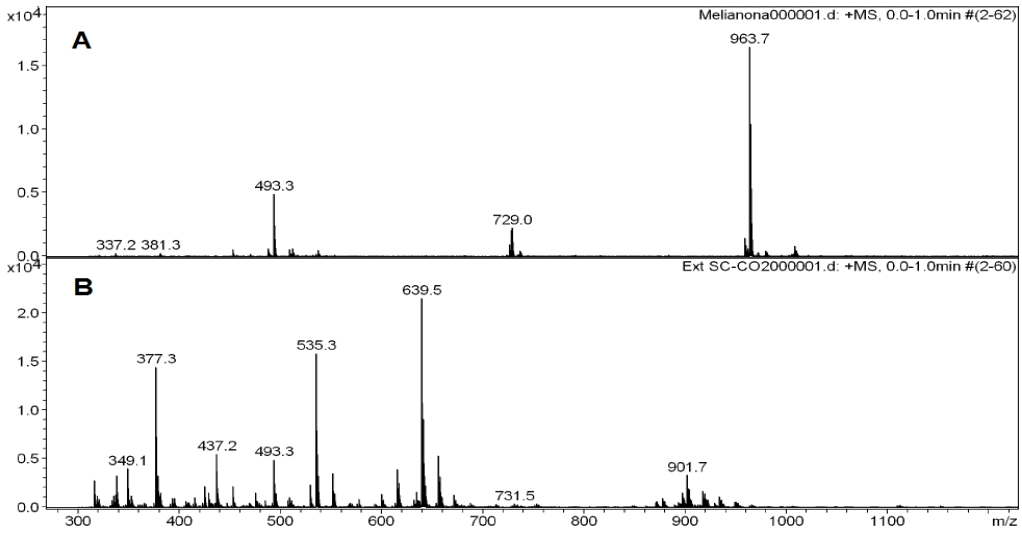

Figure 4. Mass spectrum of melianona compound (A) determined by ESI-Q-TOF-MS analysis showing ion $[M + Na]^+$ (m/z 493.3). Mass spectrum of the extract SC-CO_2 with ion mass 493.3 also present (B)

3.3 Bioassay test against *S. frugiperda*

The SC-CO_2 extract of fruits of *M. azedarach* significantly affected the survival of larvae of *S. frugiperda* at all concentrations tested, with increased mortality proportional to the increase of extract added to the diet (Table 3), with LC_{50} of 376.74mg/Kg (the concentration that produces 50% mortality). None caterpillar of treatment with 5000mg/Kg completed the larval stage. In this, the 50% of mortality of larvae was recorded in 20 days after the start of the experiment, reaching 100% in 30 days. For this same lepidopteran, the aqueous extract of leaves of *M. azedarach* caused 100% larval mortality at concentration of 10000 mg/Kg [60], and the methanol extract of leaves, 100% mortality was recorded at a concentration of 4000 mg/Kg [61], demonstrating that the SC-CO_2 extract of *M. azedarach* has insecticide effects similar, and even superior, to that of extracts obtained by conventional extraction methods.

Other effect observed was that many caterpillars in the treatments with 500, 1000 and 5000mg/Kg died during ecdysis, especially during the formation of the pupa, which is associated with a disturbance in the endocrine system of insects, causing death during the sclerotization, due to reduction and blocks the release of eclosion hormone, preventing the completion of morphogenesis [62].

Besides affecting survival, the extract of *M. azedarach* also influenced the development of the *S. frugiperda* larvae, which had mean weight and length significantly lowers than in the control treatment, what is associated with an antifeedant action of the extract, as well, is reported that extracts this meliaceous promote activation of cytochrome P-450, which constitutes an important mechanism for degradation of toxic [63-64], diverting energy resources that would normally be used for biomass gain.

Table 3. Results obtained in tests with not choice during the stages larval, pupal and moth of *S. frugiperda* after application of the SC-CO_2 extracts of *M. azedarach* at different concentrations in larvae diet

	Larval period			Pupal and adult periods				
Treatment (mg/Kg)	Weight (mg) [(1)]	Body length (cm) [(1)]	Mortality (%) [(2)]	Pupae weight (mg)	Pupation (%) [(3)]	Emergence (%) [(4)]	Moths with morphological deformities (%) [(5)]	Total viability (%) [(6)]
Control	384.40 ±87.4 [a]	3.050 ±0.30 [a]	–	384.40 ±87.4 [a]	82.5	78.8	0.0	78.8
100	379.21 ±96.4 [ab]	2.975 ±0.33 [a]	21.21	379.21 ±96.4 [ab]	65.0	57.7	26.7	27.7
500	321.38 ±123.3 [b]	2.824 ±0.38 [a]	48.48	321.38 ±123.3 [b]	42.5	64.7	9.1	25.0
1000	133.19 ±100.6 [c]	2.159 ±0.49 [b]	57.58	133.19 ±100.6 [c]	35.0	42.9	0.0	15.0
5000	29.7 ±20.3[d]	1.268 ±0.32 [c]	100.00	–	0.0	–	–	0.0

Values followed by the same letter in columns do not differ by Tukey test at significance level of 95%.
[(1)] Values recorded thirteen days after treatment
[(2)] Corrected mortality Abbott Equation (Heong et al., 1998).
[(3)] % = number of pupae ×100/total number of larvae.
[(4)] % = number of adults emerged×100/total number of pupae.
[(5)] % = number of adults with morphological deformities×100/total number of adults.
[(6)] % = number of individuals what completed all stages×100/initial number of larvae.

Smaller larvae gave rise to pupae significantly lower in the treatments with SC-CO_2 extract of *M. azedarach* (500, 1000, 5000mg/Kg), and pupae biomass has a high correlation with the fecundity of adults, being reported reduction in fecundity and the number of offspring [65]. Species such as *S. frugiperda* use foods of low nutritional quality during adulthood, and depend on the protein reserves accumulated in the larval stage. The occurrence of sterility in these insects is usually associated with eating disorders during the larval period [66].

The pupal viability was also lower in the treatments with the highest concentration of extract in the diet. The treatment with the lowest concentration of extract (100mg/Kg) showed the least adverse effects in *S. frugiperda* during larval and pupal stages, but in adulthood, this extract was which resulted in much of emerging adults with morphological deformities. Because of the normal development of insects in this treatment, food intake was normal, causing accumulation of toxic substances in the organism of these insects during the larval stage, causing retarded bioactive effect, when there was no more exposure to the extract. The morphological deformations observed in moths *S. frugiperda* were malformed wings and difficulties of insects free themselves from the pupal casing. At the end of this experiment, no treatment with added extract on diet the percentage of individuals who reached adulthood (total viability) was superior to 30%.

4. Conclusion

In the SC-CO_2 extraction of the fruits of *M. azedarach*, the variables that most influenced the extraction yield were system pressure and particle size, resulting in the best extraction yield at pressure of 250 bar and 0.85 mm particle size. The temperature however not significant affected the extraction yield but using a pressure of 250bar at 60°C resulted in highest yield. The SC-CO_2 extract of *M. azedarach* showed biological activity against *S. frugiperda* at all concentrations tested. At higher concentrations (500, 1000 and 5000mg/Kg) bioactive effects were observed during larval and pupal stages, with larvae and pupae significantly lower than the control treatment. At the lowest concentration (100mg/Kg) the extract caused retarded bioactive effect, with adults presenting morphological deformities.

Acknowledgments

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References

[1] M. Arias-Estévez, E. López-Periago, E. Martínez-Carballo, J. Simal-Gándara, J. Mejuto, L. García-Río, The mobility and degradation of pesticides in soils and the pollution of groundwater resources, Agriculture, Ecosystems and Environment 123 (2008) 247–260.

[2] J. Demajorovic, Sociedade de risco e responsabilidade socioambiental: perspectivas para a educação corporativa. São Paulo, Senac São Paulo, 2003.

[3] M.B. Isman, Tropical forests as sources of natural insecticides, in: J. T. Romeo (Ed.), Chemical ecology and phytochemistry of forest ecosystems, Recent advances in phytochemistry 39, 2005, pp. 145-161.

[4] E. Reverchon, I. De Marco, Supercritical fluid extraction and fractionation of natural matter, J. Supercritical Fluids 38 (2006) 146–166.

[5] L. SHI, Z. Zang, L. Xing, D. Yang, Y. Guo, X. Guo, L. Zhao, Z. Tang, Antioxidants extraction by supercritical CO_2, J. Medicinal Plants Research 5 (2011) 300 – 308.

[6] A. Strattmann, G. Schweiger, Fluid phase equilibrium of binary mixtures whit supercritical solvents with in-situ concentration measurements by Raman spectroscopy, in: G. Brunner (Ed.), Supercritical fluids as solvents and reaction media, Netherlands, Elsevier, 2004, p. 85–120.

[7] I.L. Panza, E.J. Beckman, Chemistry and materials desing for CO_2 processing, in: P. York, U.B. Kompella, B.Y. Shekunov (Eds.), Supercritical fluid technology for drug product development 138, Taylor-Francis e-Library, 2004, p. 1–23.

[8] J.A. Mendiola, M. Herrero, A. Cifuentes, E. Ibãnez, Use of compressed fluids for sample preparation: food applications, J. Chromatography A 1152, (2007) 234– 246.

[9] P. Ambrosino, R. Fresa, V. Fogliano, S.M. Monti, A. Ritieni, Extraction of azadirachtin A from neem seed kernels by supercritical fluid and its evaluation by HPLC and LC/MS, J. Agricultural and Food Chemistry 47 (1999) 5252–5256.

[10] P. Tonthubthimthong, P.L. Douglas, S. Douglas, W. Luewisutthichat, W. Teppaitoon, L. Pengsopa, Extraction of nimbin from neem seeds using supercritical CO_2 and a supercritical CO_2 – methanol mixture, J. Supercritical Fluids 30 (2004) 287–301.

[11] S. Ismadji, Y. Ju, F.E. Soetaredjo, A. Ayucitra, Solubility of azadirachtin in supercritical carbon dioxide at several temperatures, J. Chemical & Engineering Data 56 (2011) 4396–4399.

[12] M.C. Carpinella, M.T. Defago, G. Valladares, S.M. Palacios, Role of *Melia azedarach* L. (Meliaceae) for the control of insects and acari: present status and future prospect, in: M.C. Carpinella, M. Rai (Eds.), Naturally Occurring Bioactive Compounds, Amsterdam, Elsevier, 2006, p. 81–123.

[13] M.T. Defagó, G. Valladares, E. Banchio, C. Carpinella, S. Palacios, Insecticide and antifeedant activity of different

plant parts of *Melia azedarach* on *Xanthogaleruca luteola*, Fitoterapia 77 (2006) 500–505.
[14] SS. Nathan, Effects of Melia azedarach on nutritional physiology and enzyme activities of the rice leaffolder Cnaphalocrocis medinalis (Guenée) (Lepidoptera: Pyralidae), Pesticide Biochemistry and Physiology 84 (2006) 98–108.
[15] M.M.O. Cabral, E.R.F. Crescente, P.M. Mendonça, C.M.S. Gomes, V.C. Oliveira, A. Kelecom, *Melia azedarach* L. extracts and their activity on *Musca domestica* L. (Diptera: Muscidae), Brazilian J. Pharmacognosy 18 (2008) 699–702.
[16] J.S. Prophiro, J.C.N. Rossi, M.F. Pedroso, L. Kanis, O.S. Silva. Leaf extracts of *Melia azedarach* Linnaeus (Sapindales: Meliaceae) act as larvicide against *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae), Revista da Sociedade Brasileira de Medicina Tropical 41 (2008) 560–564.
[17] M.T. Defagó, A. Dumón, D.S. Avalos, S.M. Palacios, G. Valladares, Effects of *Melia azedarach* extract on *Cotesia ayerza*, parasitoid of the alfalfa defoliator *Colias lesbia*, Biological Control 57 (2011) 75–78.
[18] J.D. Holloway, H.S. Barlow, Potential for loss of biodiversity in Malaysia, illustrated by the moth fauna, in: A. Aziz, S.A. Kadir, H.S. Barlow (Eds.), Pest management and the environment in 2000, Wallingford, CAB International & Agricultural Institute of Malaysia, 1992, p. 293–311.
[19] Cruz, I. A lagarta-do-cartucho na cultura do milho. Sete Lagoas, Embrapa – CNPMS, Circular técnica 21, 1995.
[20] G.R. Busato, A.D. Grützmacher, M.S. Garcia, M.S. Giolo, G.J. Stefanello Jr., M.J. Zotti, Feeding preference of the fall armyworm biotypes for corn, sorghum, rice and barnyardgrass, Revista Brasileira de Agrociência 10 (2004) 215–218.
[21] A. Specht, J.A. Teston, R.A. Di Mare, E. Corseuil, Owlet moths (Lepidoptera, Noctuidae) collected in four protected areas located in Rio Grande do Sul State, Brazil, Revista Brasileira de Entomologia 49 (2005) 130–140.
[22] M.R.A. Rodrigues, E.B. Caramão, J.G. Santos, C. Dariva, J.V. Oliveira, The effects of temperature and pressure on the characteristics of the extracts from high-pressure CO_2 extraction of *Majorana hortensis* Moench. J. Agricultural and Food Chemistry 51 (2003) 453-456.
[23] A.J. Mossi, R.L. Cansian, A.Z. Carvalho, C. Dariva, J.V. Oliveira, M. Mazutti, I. Nascimento Filho, S. Echeverrigaray, Extraction and characterization of volatile compounds in *Maytenus ilicifolia* Mart. ex Reiss, using high-pressure CO_2. Fitoterapia 75 (2004) 168-178.
[24] M.R.A. Rodrigues, L.C. Krause, E.B. Caramão, J.G. Santos, C. Dariva, J.V. Oliveira, Chemical Composition and Extraction Yield of the Extract of *Origanum vulgare* Obtained from Sub- and Supercritical CO_2. J. Agricultural and Food Chemistry 52 (2004) 3042-3047.
[25] R.A. Jacques, L.C. Krause, L.S. Freitas, C. Dariva, J.V. Oliveira, E.B. Caramão, Influence of drying methods and agronomic variables on the chemical composition of mate tea leaves (*Ilex paraguariensis* A. St.-Hil) obtained from high-pressure CO_2 extraction. J. Agricultural and Food Chemistry 55 (2007) 10081-10085.
[26] J. Yu, D.V. Dandekar, R.T. Toledo, R.K. Singh, B.S. Patil, Supercritical fluid extraction of limonoids and naringin from grapefruit (*Citrus paradisi* Macf.) seeds. Food Chemistry 105 (2007) 1026–1031.
[27] J.B. Harbore, Phytochemical methods: A guide to modern techniques of plant analysis, 3rd ed., Chapman & Hall, 1998.
[28] G.L. Greene, N.C. Leppla, W.A. Dickerson, Velvetbean caterpillar: a rearing procedure and artificial medium. J. Economic Entomology 69 (1976) 487–488.
[29] K.L. Heong, K.H. Tan, C.P.F. Garcia, L.T. Fabellar, Z. Lu, Research methods in toxicology and insecticide resistance monitoring of rice planthoppers, Philippines, International Rice Research Institute, 2011, p. 37–42.
[30] E.W.Z. Michielin, L.F.V. Bresciani, L. Danielski, R.A. Yunes, S.R.S. Ferreira, Composition profile of horsetail (*Equisetum giganteum* L.) oleoresin: comparing SFE and organic solvents extraction. J. Supercritical Fluids 33 (2005) 131–138.
[31] K.L. Nyan, C.P. Tan, O.M. Lai, K. Long, Y.B. Che Man, Optimization of supercritical fluid extraction of phytosterol from roselle seeds with a central composite design model, Food and Bioproducts Processsing 88 (2010) 239–246
[32] P. Benelli, C.A.S. Riehl, A. Smânia Jr., E.F.A. Smânia, S.R. S. Ferreira, Bioactive extracts of orange (*Citrus sinensis* L. Osbeck) pomace obtained by SFE and low pressure techniques: Mathematical modeling and extract composition. J. Supercritical Fluids 55 (2010) 132–141.
[33] B.D. Drake, R.L. Smith Jr., Measurement of static dielectric constants of supercritical fluid solvents and cosolvents: Carbon dioxide and argon, carbon dioxide, and methanol at 323 K and pressures to 25 MPa. J. Supercritical Fluids 3, (1990) 162-168.
[34] M.B. King, T.R. Bott (Eds.), Extraction of natural products using near-critical solvents, Great Britain, Blackie Academic & Professional, Chapman Hall, 1993, p. 1–33.
[35] M.A. McHugh, V.J. Krukonis, Supercritical Fluid Extraction: Principles and practice, Butterworth-Heinemann, 1994, p. 404.
[36] S.M. Pourmortazavi, S.S. Hajimirsadeghi, Supercritical fluid extraction in plant essential and volatile oil analysis, J. Chromatography A 1163 (2007) 2–24.
[37] Z. Berck, Food process engineering and technology, Food Science and Tecnology International Series, Elsevier, 2009, p. 271–277.
[38] P.G. Jessop, W. Leitner (Eds.), Chemical synthesis using supercritical fluids. New York, Wiley-VCH, 1999, p. 37–

53.
[39] S. Machmudah, M. Kondo, M. Sasaki, M. Goto, J. Munemasa, M. Yamagata, Pressure effect in supercritical CO_2 extraction of plant seeds, J. Supercritical Fluids 44 (2008) 301–307.
[40] Y. Tong, L. Gao, G. Xiao, X. Pan, Supercritical CO_2 extraction of Chlorophyll a from *Spirulina platensis* with a static modifier, Chemical Engineering Technology 34, (2011) 241–248.
[41] K. Ansari, I. Goodarznia, Optimization of supercritical carbon dioxide extraction of essential oil from spearmint (*Mentha spicata* L.) leaves by using Taguchi methodology, J. Supercritical Fluids (2012) 123–130.
[42] D. Tuma, G.M. Schneider, High-pressure solubility of disperse dyes in near and supercritical fluids: measurements up to 100 MPa by a static method. J. Supercritical Fluids 13 (1998) 37–42.
[43] A. Sheibani, H. S. Ghaziaskar, Pressurized fluid extraction of pistachio oil using a modified supercritical fluid extractor and factorial design for optimization, LWT - Food Science and Technology 41 (2008) 1472–1477.
[44] A. Ajchariyapagorn,T. Kumhom, S. Pongampha, S. Douglas, P.L. Douglas, W. Teppaitoon, Predicting the extraction yield of nimbin from neem seeds in supercritical CO_2 using group contribution methods, equations of state and a shrinking core extraction mode, J. Supercritical Fluids 51 (2009) 36–42.
[45] J.M. Del Valle, E.L. Uquiche, Particle size effects on supercritical CO2 extraction of oil-containing seeds. J. American Oil Chemists' Society 79 (2002) 1261–1266.
[46] K. Araus, E.L. Uquiche, J.M. Del Valle, Matrix effects in supercritical CO_2 extraction of essential oils from plant material, J. Food Engineering 92 (2009) 438–447.
[47] S.R.S. Ferreira, M.A.A. Meireles, Modeling the supercritical fluid extraction of black pepper (*Piper nigrum* L.) essential oil, J. Food Engineering 54 (2002) 263–269.
[48] S.R.S. Ferreira, M.A.A. Meireles, F.A. Cabral, Extraction of essential oil of black pepper with liquid carbon dioxide, J. Food Engineering 20 (1993) 121–133.
[49] M.A.A. Meireles, Supercritical extraction from solid: process design data (2001–2003), Current Opinion in Solid State and Materials Science 7 (2003) 321–330.
[50] I. Papamichail, V. Louli, K. Magoulas, Supercritical fluid extraction of celery seed oil. J. Supercritical Fluids 18 (2000) 213–226.
[51] S.A. Aleksovski, H. Sovová, Supercritical CO_2 extraction of *Salvia officinalis* L., J. Supercritical Fluids 40 (2007) 239–245.
[52] I. Zizovic, M. Stamenic, J. Ivanovic, A. Orlovic, M. Ristic, S. Djordjevic, S.D. Petrovic, D. Skala, Supercritical carbon dioxide extraction of sesquiterpenes from valerian root, J. Supercritical Fluids 43 (2007) 249–258.
[53] E.K. Asep, S. Jinap, T.J. Tan, A.R. Russly, S. Harcharan, S.A.H. Nazimah, The effects of particle size, fermentation and roasting of cocoa nibs on supercritical fluid extraction of cocoa butter. J. Food Engineering 85 (2008) 450–458.
[54] B. Nagy, B. Simándi, Effects of particle size distribution, moisture content, and initial oil content on the supercritical fluid extraction of paprika. J. Supercritical Fluids 46 (2008) 293–298.
[55] D. Mongkholkhajornsilp, S. Douglas, P.L. Douglas, A. Elkamel, W. Teppaitoon, S. Pongamphai, Supercritical CO_2 extraction of nimbin from neem seeds – a modelling study, J. Food Engineering 71 (2005) 331–340.
[56] G. Zahedi, A. Elkamel, A. Lohi, T. Hatami, Optimization of supercritical extraction of nimbin from neem seeds in presence of methanol as co-solvent, J. Supercritical Fluids 55 (2010) 142–148.
[57] J. Polonsky, Z. Varon, R.M. Rabanal, 21,20-Anhydromelianone and melianone from *Simarouba amara* (Simaroubaceae); Carbon-13 NMR spectral analysis of Δ^7-Tirucallol-Type triterpenes, Israel Journal of Chemistry 16 (1977) 16–19.
[58] W.L. Wang, Y. Wang, S.F. Chiu, The toxic chemical factors in the fruits of *Melia azedarach* and their bio-activities toward *Pieris rapae*, Acta Entomologica Sinica 37 (1994) 20–24.
[59] R.H. Su, M.K. Takehiki, S. Takanashi, Antifeeding constituents of Phellondendron chinese fruit against Reticulitermes speratus, J. Pesticide Science 15 (1990) 567–572.
[60] C.H. Brito, J.A. Mezzomo, J.L. Batista, M.S.B. Lima, A.T. Murata, Bioactivity of botanical aqueous extracts on *Spodoptera frugiperda* under laboratory conditions. Manejo Integrado de Plagas y Agroecología 71 (2004) 41–45.
[61] V. Bullangpoti, E. Wajnberg, P. Audant, R. Feyereisen, Antifeedant activity of *Jatropha gossypifolia* and *Melia azedarach* senescent leaf extracts on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and their potential use as synergists. Pest Management Science 68 (2012) 1255–1264.
[62] C.L. Céspedes, J.R. Salazar, M. Martínez, E. Aranda, Insect growth regulatory effects of some extracts and sterols from *Myrtillocactus geometrizans* (Cactaceae) against *Spodoptera frugiperda* and *Tenebrio molitor*, Phytochemistry 66 (2005) 2481–2493.
[63] M. Breuer, B. Hoste, A.D. Loof, S.N.H. Naqvi, Effect of *Melia azedarach* extract on the activity of NADPH-cytochrome c reductase and cholinesterase in insects. Pesticide Biochemistry and Physiology 76 (2003) 99–103.
[64] V. Bullangpoti, E. Wajnberg, P. Audant, R. Feyereisen, Antifeedant activity of *Jatropha gossypifolia* and *Melia azedarach* senescent leaf extracts on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and their potential use as synergists, Pest Management Science 68 (2012) 1255–1264.
[65] [65] M. Botton, J.J. Carbonari, M.S. Garcia, J.F.S. Martins, Feeding preference and biology of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on rice and barnyardgrass, Anais da Sociedade Entomológica do Brasil 27,(1998) 207–212.

[66] E.L.N. Costa, R.F.P. Silva, L.M. Fiuza, Effects, uses and restrictions of insecticidal plants extracts. Acta Biologica Leopoldensia 26 (2004) 173–185.