

CHEMICAL CONSTITUENTS OF METHANOLIC EXTRACTS OF *Jatropha curcas* L AND EFFECTS ON *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)#

Sandra Santos Ribeiro, Thanany Brasil da Silva, Valéria Regina de Souza Moraes, Paulo Cesar de Lima Nogueira e Emmanoel Vilaça Costa

Departamento de Química, Universidade Federal de Sergipe, 49100-000 São Cristóvão – SE, Brasil

Antônio Rogério Bernardo, Andreia Pereira Matos, João Batista Fernandes* e Maria Fátima das Graças Fernandes da Silva

Departamento de Química, Universidade Federal de São Carlos, 13565-905 São Carlos – SP, Brasil

Ângela Maria dos Santos Pessoa e Renata Silva-Mann

Departamento de Engenharia Agrônômica, Universidade Federal de Sergipe, 49100-000 São Cristóvão – SE, Brasil

Recebido em 14/5/12; aceito em 5/8/12; publicado na web em 26/10/12

The biological activity of seven extracts from leaves of different *Jatropha curcas* L (Euphorbiaceae) accessions was evaluated on *Spodoptera frugiperda*. Methanol extracts were incorporated into an artificial diet and offered to the larval stage of *S. frugiperda*. The parameters evaluated were length of larval and pupal stages, mortality of larval and total cycle stage, and weight of pupae. The extracts of the EMB accessions showed the best result for larval mortality at 60.00 and 56.67%, compared with the control, respectively. Hexane partition of the methanol extract of the leaves of PM-14 accessions allowed the identification of phytosterols, phytol and n-alkanols.

Keywords: *Jatropha curcas*; fall armyworm; insecticide activity.

INTRODUCTION

The fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is the most prevalent pest in corn crops and can reduce corn production by up to 34%, depending at which cultivation growth stage the attack occurs.¹ Fall armyworm larvae are usually controlled using pesticides such as piretroids and organophosphates when defoliation is noted in the field.² However, there are many problems associated with the use of pesticides, mainly the possibility of resistance, reduction of insect predators and parasitoids that interfere in natural control.¹ New alternatives must be pursued including botanical insecticides. These insecticides of botanical origin may represent an effective alternative to persistent synthetic insecticides, since their advantages are low mammalian toxicity, lack of neurotoxic action, low persistence in the environment and high biodegradability.³

The family Euphorbiaceae contain toxic compounds that can be used as insecticides.⁴ The *Jatropha curcas* species (Malpighiales: Euphorbiaceae) is a drought-resistant shrub that is well adapted to arid and semi-arid conditions, being a viable plant for family agriculture in the North and Northeast regions of Brazil. Furthermore, this plant has a high agro-industrial potential because the oil extracted from its seeds can be used for biodiesel production.⁵

The seeds of *J. curcas* are toxic to both humans and animals precluding their use in foodstuffs.⁶ This toxicity of the seed is attributed predominantly to the phorbol-type diterpene esters which they contain at high concentrations.⁷ Previous studies on leaves of this plant have investigated the isolation of flavonoids,⁸ sterols,⁹ polyphenols,¹⁰ besides saprophytic fungal activity,¹¹ larvicidal activity,¹² and molluscicidal activity.¹³ As a part of an ongoing study toward biorational control of *S. frugiperda*,¹⁴ the aim of the present work was to chemically investigate and evaluate the insecticidal activity

of extracts from the leaves of *J. curcas* accession (bout) against the larvae of fall armyworm under laboratory conditions.

EXPERIMENTAL

Botanical material

Leaves of six accessions, named PM-2, PM-7, PM-10, PM-11, PM-12 and PM-14, of *Jatropha curcas* L. were collected in January 2009 (for fresh leaf studies) and March 2009 (for air-dried leaf studies), from the Germoplasm Bank of the Agronomic Engineering Department of the Federal University of Sergipe, São Cristóvão, Brazil. Leaves of an accession of a *J. curcas* specimen (called EMB) were collected in October 2008, from the Experimental Campus of the “Embrapa Tabuleiros Costeiros”, in the Municipality of Umbaúba, Sergipe, Brazil. A voucher specimen (JC014URVES) was deposited at the Federal University of Sergipe Herbarium in the Biology Department.

Preparation of the extracts

Leaves, air-dried by 5 days, and fresh leaves of each *J. curcas* accession were triturated and extracted separately at room temperature with methanol (1:4 w/v) by maceration for 5 days. The solutions obtained were filtrated through analytical filter papers and solvents removed under reduced pressure to give the correspondent crude extracts of fresh and air-dried leaves (Table 1). The extraction steps for each accession were repeated twice.

Isolation and identification of the chemical constituents

Part of the crude leaf methanol extract (5.02 g) from PM-14 *J. curcas* accession was suspended in 150 mL of a solution containing MeOH:H₂O (9:1) and extracted successively with hexane (3 x 150 mL), CH₂Cl₂ (2 x 150 mL), and EtOAc (3 x 100 mL) yielding 0.67, 0.29 and 0.11 g of the respective partitions and the hydroalcoholic

*e-mail: djbf@ufscar.br

#Artigo em homenagem ao Prof. Otto R. Gottlieb (31/8/1920-19/6/2011)

Table 1. Methanolic extracts prepared from fresh and dried leaves of *J. curcas* accessions

Accession	Fresh leaves		Dried leaves	
	Plant mass (g)	Extract mass (g)	Plant mass (g)	Extract mass (g)
PM-2	227.7	10.7	123.8	10.9
PM-7	87.7	4.6	123.7	4.6
PM-10	179.0	5.1	242.1	12.6
PM-11	271.8	8.7	149.3	9.4
PM-12	249.2	7.8	134.3	9.4
PM-14	233.0	3.7	185.7	9.8
EMB	83.4	2.6	127.5	6.3

extract (1.35 g), after solvent evaporation. Part of this hexane partition (0.4 g) was subjected to silica gel column chromatography, eluting with hexane, EtOAc and MeOH in a gradient manner to yield thirty-eight subfractions. The eleventh fraction (31.9 mg) was further purified by preparative thin-layer chromatography (TLC) with hexane-EtOAc (90:10, v/v) and 10 drops of MeOH as the mobile phase to yield the fraction 1 (14.9 mg). Following the same procedure, the seventh fraction (20.0 mg) was further purified by preparative thin-layer chromatography (TLC) with hexane-EtOAc (90:10, v/v) as the mobile phase to yield fraction 2 (4 mg).

Fractions 1 and 2 were investigated by gas chromatography-mass spectrometry (GC/MS) and the compounds **1-3** (fraction 1) and **4-7** (fraction 2) were identified in these fractions. Analysis was carried out on a Shimadzu QP5050A device with a capillary column DB5-MS (30 m x 0.25 mm, 0.25 μ m film thickness). Temperature program: 180 °C (held for 1 min) to 300 °C at a heating rate of 20 °C min⁻¹ (held for 5 min), then raised from 300 to 320 °C at a heating rate of 5 °C min⁻¹ (held for 10 min). Helium was used as the carrier gas. The interface was set at 280 °C and the ionization voltage was 70 eV (electron impact mode). Fractions 1 and 2 were also analyzed by ¹H and ¹³C NMR spectroscopy on a BRUKER DRX 400 device using CDCl₃ as a solvent and TMS as an internal standard.

Derivatization

For the silylation reaction, 100 μ L of N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA, >99% Sigma-Aldrich) was added to fraction 2 and then left at room temperature (~30 °C) for 1 h. The residue was taken up in 250 μ L of dichloromethane and then analyzed by GC/MS. The identification of silyl derivatives was performed by comparison with reported mass spectra, interpretation of fragmentation patterns and gas chromatographic retention indices.^{15,16}

Biological bioactivity

Larvae of *S. frugiperda* were obtained from the Insect Bioassay Laboratory of the Federal University of São Carlos, Brazil, and reared on artificial diets.^{17,18} They were maintained in an incubation chamber with a light phase of 12:12 h L:D, 70 \pm 5% relative humidity and temperature of 25 \pm 1 °C. For each treatment and control, 30 neonate larvae of *S. frugiperda* were used. The methanolic extracts were added to ascorbic acid (1.56 g; an ingredient of the diet). After evaporation, the mixture was incorporated into the artificial diet in which bean and wheat germ were the basic ingredients¹⁷ at a final content of 1000 mg kg⁻¹ for the methanolic extract of *J. curcas*. The diet for the control larvae was prepared similarly but with no extract. The diets were placed in previously sterilized glass tubes (8.5 cm x 2.5 cm) into which

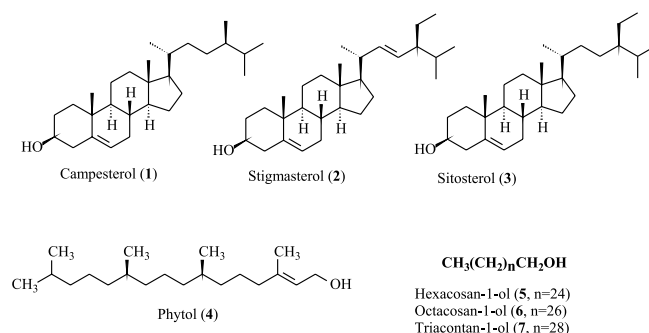
larvae of *S. frugiperda* were introduced individually. The resultant pupae were weighed after pupation and transferred into plastics cups, where they were kept until the emergence of adults. Daily observations were made and the following parameters evaluated: duration of larval and pupal phases; weight of pupae and percentage of dead insects (mortality) at the end of each phase.

Statistics

Data were submitted to analysis of variance ANOVA¹⁹ and averages were compared by applying the Tukey test ($P \leq 0.05$). Each tube containing one insect, independent of the development phase, was considered as one replicate therefore the number of replicates was different for each treatment. For evaluation of the mortality of the larval and pupal phases, the experimental unit constituted the mean of five tubes containing one larva each, with thirty replications per treatment.

RESULTS AND DISCUSSION

The analysis of fraction 1 by ¹H and ¹³C NMR²⁰ and GC/MS²¹ led to the identification of a mixture of the three known phytosterols campesterol (**1**), stigmasterol (**2**) and β -sitosterol (**3**) (Figure 1), which were similar to those found by Hufford and Oguntimein.⁹

**Figure 1.** Compounds isolated from leaves of *Jatropha curcas* L.

The identification of compounds from fraction 2 was based on characteristic mass spectra fragmentation patterns of trimethylsilyl (TMS) ether derivatives of the phytol (**4**) and alcohols, by comparing the retention times and mass spectrum of an authentic sample. The EI spectra of their TMS derivatives were identical to those of phytol (**4**), hexacosan-1-ol (**5**), octacosan-1-ol (**6**) and triacontan-1-ol (**7**) (Figure 1), respectively as was their retention index, generated with a standard solution of n-alkanes (C₉-C₄₀).^{15,22}

Methanolic extractions were conducted with fresh and air-dried leaves (Table 1). Among the extracts of fresh leaf accessions tested, only PM-12 and PM-14 revealed a statistical difference in relation to the control. There was a shortening of 2.80 days in larval stage for PM-12 and 1.06 days in pupal stage for PM-14, in comparison with the control treatment, whose larval and pupal stages were 21.70 and 11.77 days, respectively. No statistically significant changes in pupal weight and mortality were found in relation to the control (data not shown). The EMB accession had the highest mortality in the larval stage (60%) compared to the control while the other accessions showed from moderate (16.66 to 13.33%) to low activity (10.00 to 3.33%, Table 2).

The period of larval stage submitted to methanolic extracts of air-dried leaves of the *J. curcas* accessions showed statistically moderate difference for PM-2, PM-7, PM-10 and PM-11, being 2.44, 2.18 and 2.49 days shorter than the control, respectively. Pupal period was similar to the control whereas pupal mortality showed no statistically

Table 2. Mean of larval and pupal period and larval mortality of *S. frugiperda* fed on artificial diet treated with bouts of fresh leaves, 1000 mg kg⁻¹, of *J. curcas*. Temp.: 25 ± 1 °C; UR: 70 ± 5% and photophase: 12 h

Treatment		Larval period (days) (±EP) ¹	Pupal period (days) (±EP) ¹	Larval mortality (%) (±EP) ¹
<i>Jatropha curcas</i> Fresh leaves	PM-2	20.00 ± 1.95 bc	11.70 ± 1.03 ab	3.33 ± 5.77 b
	PM-7	19.00 ± 0.91 bc	11.54 ± 1.14 ab	10.00 ± 10.00 b
	PM-10	20.38 ± 2.80 b	11.64 ± 0.90 ab	13.33 ± 5.77 b
	PM-11	19.33 ± 1.04 bc	11.62 ± 1.13 ab	10.00 ± 10.00 b
	PM-12	18.90 ± 1.01 c	11.04 ± 1.14 ab	3.33 ± 5.77 b
	PM-14	20.00 ± 1.19 bc	10.71 ± 1.40 b	16.67 ± 15.28 b
	EMB	22.58 ± 2.54 a	11.91 ± 1.22 ab	60.00 ± 20.00 a
Control		21.70 ± 2.15 ab	11.77 ± 0.99 a	0.00 ± 0.00 b

¹ Means followed by same letter in column do not differ by Tukey test (P ≤ 0.05).

Table 3. Mean of larval and pupal period and larval mortality of *S. frugiperda* fed on artificial diet treated with bouts of air-dried leaves, 1000 mg kg⁻¹, of *J. curcas*. Temp.: 25 ± 1 °C; UR: 70 ± 5% and photophase: 12 h

Treatment		Larval period (days) (±EP) ¹	Pupal period (days) (±EP) ¹	Larval mortality (%) (±EP) ¹
<i>Jatropha curcas</i> Air-dried fresh	PM-2	19.26 ± 2.28 b	10.96 ± 1.10 b	10.00 ± 10.00 bc
	PM-7	19.52 ± 1.47 b	11.71 ± 2.15 ab	13.33 ± 15.27 b
	PM-10	19.26 ± 1.13 b	12.27 ± 1.23 a	30.00 ± 0.00 b
	PM-11	19.21 ± 1.64 b	11.81 ± 1.33 ab	10.00 ± 0.00 bc
	PM-12	20.33 ± 1.15 ab	11.15 ± 1.29 ab	6.67 ± 5.77 c
	PM-14	22.15 ± 2.03 a	11.12 ± 1.25 ab	60.00 ± 10.00 a
	EMB	19.52 ± 1.47 b	11.36 ± 1.21 a	56.67 ± 5.77 a
Control		21.70 ± 2.15 a	11.77 ± 0.99 ab	0.00 ± 0.00 c

¹ Means followed by same letter in column do not differ by Tukey test (P ≤ 0.05).

significant difference. The larval mortality was higher for accessions EMB, PM-14 and PM-10 (56.67, 60.00 and 30.00%, respectively), compared to the control (0.00%) (Table 3).

In a previous study, Devanand and Rani tested ketone extracts of leaves of *J. curcas* and other species of Euphorbiaceae, Myrtaceae and Rutaceae families.²³ Their results have proven that the species *J. curcas* has a significant anti-feeding activity in *Achaea janata* L. (greater than 90%) and moderate in *Spodoptera litura* Fab. (52%). The mean (%) toxicity ± SD of this extract was 36.6 ± 4.7 and 60.6 ± 60.6 in *S. litura* and *A. janata*, respectively and LD₅₀ (95% CL, mg/ 21cm²/ larva) were higher than 100 for both assays.²³ Based on the results of the cited study and activity observed in the present work, *J. curcas* can be classified as a good alternative for integrated pest management due to its toxic and antifeeding properties for several species of pests and minimum environmental impact. Thorough chemical fractionation of active extract and development of a methodology to apply crude extract of *J. curcas* are underway and is hoped to reveal some bioactive compounds and a methodology for using crude extract to control plague insects.

ACKNOWLEDGEMENTS

This research received partial support from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) - (Brazilian government entity promoting the scientific and technological development), FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), FAPITEC/SE (Fundação de Apoio à Pesquisa e à Inovação Tecnológica do Estado de Sergipe), and CAPES/PROCAD. The authors are grateful to Prof. Dr. M. da R. Alexandre (DQI/UFS) who kindly provided the BSTFA reagent for silylation and a sample of

n-alkanes for the retention index calculation.

REFERENCES

- Cruz, I.; *Sete Lagoas*, Embrapa/CNPMS, 1995, Circular Técnica, n. 21.
- Jesus, F. L.; Boiça Jr., A. L. In *Tópicos em Entomologia Agrícola II*; Silva, A. G.; Rodrigues, C. A.; Becaro, C. K.; Bottega, D. B.; Haddad, G. K.; Alvez, G. C. S.; Janini, J. C., eds.; Funep: Jaboticabal, 2009.
- Céspedes, C. L.; Calderón, J. S.; Lina, L.; Aranda, E.; *J. Agric. Food Chem.* **2000**, *48*, 1903; Céspedes, C. L.; Salazar, J. R.; Martínez, M.; Aranda E.; *Phytochemistry* **2005**, *66*, 2481.
- Cruz, I.; Viana, P. A.; Waquil, J. M.; *Sete Lagoas*, Embrapa/CNPMS, 2002, Comunicado Técnico, n. 49.
- Jain, P. S.; Sharma, M. P.; *Ren. Sust. Energ. Rev.* **2010**, *14*, 3140; Suarez, P. A. Z.; Santos, A. L. F.; Rodrigues, J. P.; Alves, M. B.; *Quim. Nova* **2009**, *32*, 768; Alves, J. M. A.; Sousa, A. A.; da Silva, S. R. G.; Lopes, G. N.; Smiderle, O. J.; Uchôa, S. C. P.; *Rev. Agroambiente on-line* **2008**, *2*, 57.
- Ahmed, W. A.; Salimon, J.; *Eur. J. Sci. Res.* **2009**, *31*, 429.
- Haas, W.; Sterk, H.; Mittelbach, M.; *J. Nat. Prod.* **2002**, *65*, 1434.
- Abd-Alla, H. I.; Moharram, F. A.; Gaara, A. H.; El-Safy, M. M. Z.; *Naturforsch., C: J. Biosci.* **2009**, *64*, 495; Ebuehi, O. A. T.; Okorie N. A.; *Niger Q. J. Hosp. Med.* **2009**, *19*, 200.
- Hufford, C. D.; Oguntimein, B. O.; *Lloydia* **1978**, *41*, 161.
- Rideout, J. A.; Ragasa, C.; Ngo, H. T.; *AGGC Chem. Res. Commun.* **2003**, *16*, 34.
- Ayanbembe, G. M.; Ojo, T. K.; Afolabi, E.; Opara, F.; Orsaah, S.; Ojerinde, O. S.; *J. Clin. Lab. Anal.* **2009**, *23*, 161.
- Rahuman, A. A.; Gopalakrishnan, G.; Venkatesan, P.; Geetha, K.; *Parasitol. Res.* **2008**, *102*, 867.

13. Wang, Z. Y.; Du, Y. Q.; Qin, Y. Z.; Chen, J. F.; Quan, Z. M.; *J. Southern Medical University* **2009**, *29*, 1235.
14. Matos, A. P.; Leite, A. C.; Batista-Pereira, L. G.; Vieira, P. C.; Fernandes, J. B.; Da Silva, M. F. G. F.; *Z. Naturforsch., C: J. Biosc.* **2009**, *64*, 441.
15. Matos, A. P.; Nebo, L.; Vieira, P. C.; Fernandes, J. B.; Silva, M. F. G. F.; Rodrigues, R. R.; *Quim. Nova* **2009**, *32*, 1553.
16. van den Dool, H.; Kratz, P. D.; *J. Chromatogr.* **1963**, *11*, 463; Lytovchenko, A.; Beleggia, R.; Schauer, N.; Isaacson, T.; Leuendorf, J. E.; Hellmann, H.; Rose, J. K. C.; Fernie, A. R.; *Plant Methods* **2009**, *5*, 4.
17. Lazari, D. M.; Skaltsa, H. D.; Constantinidis, T.; *Flavour Fragr. J.* **2000**, *15*, 7.
18. Kasten, P. Jr.; Precetti, A. A. C. M.; Parra, J. R. P.; *Rev. Agric.* **1978**, *53*, 68.
19. Parra, J. R. P. In *Controle microbiano de insetos*; Alves, S. B., ed.; São Paulo, Editora Manole: São Paulo, 1986, p. 348-373.
20. Zar, J. H.; *Biostatistical Analysis*, Prentice-Hall Inc.: Englewood Cliffs, 1984.
21. Facundo, V. A.; Polli, A. R.; Rodrigues, R. V.; Militão, J. S. L. T.; Stabelli, R. G.; Cardoso, C. T.; *Acta Amazonica* **2008**, *38*, 733; Jain, P. S.; Bari S. B.; *Asian J. Plant Sci.* **2010**, *9*, 163.
22. Moccelini, S. K.; da Silva, V. C.; Ndiaye, E. A.; de Souza Jr., P. T.; *Quim. Nova* **2009**, *32*, 131.
23. Christie, W. W.; *Gas Chromatography and Lipids: a practical guide*, The Oily Press.: Bridgwater, 1989.
24. Devanand, P.; Rani, P. U.; *J. Biopest.* **2008**, *1*, 170.