

Effects of plant extracts on developmental stages of the predator *Podisus nigrispinus* (Hemiptera: Pentatomidae)

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Podisus species (Hemiptera: Pentatomidae) are generalist predators that survive and reproduce in temporary agro-ecosystems. They have large numbers of offspring per generation (Oliveira et al. 2002) and adapt to various temperatures and prey (Torres et al. 1998; Lemos et al. 2003; Vivan et al. 2003). Such predators represent an alternative method of controlling insect pests in integrated management programs (Matos-Neto et al. 2002; Lemos et al. 2005).

The predator *Podisus nigrispinus* (Dallas) is one of the most common species of Asopinae in the Neotropics and has been reported in several countries in Central and South America (Matos-Neto et al. 2002; Silva et al. 2009). However, *P. nigrispinus* preys on insect defoliators and can be exposed to toxic compounds directly and indirectly via its prey (Torres et al. 2003, 2010; Torres & Ruberson 2004).

Synthetic insecticides can be harmful to the environment and natural enemies (Silva et al. 2005; Campiche et al. 2006; Rocha et al. 2006), making it necessary to select those which are effective, safe, and selective for pest control. Botanical extracts are a potentially valuable alternative method of controlling insect pests as they have lower persistence and toxicity than synthetic insecticides (Wiesbrook 2004; Isman 2006; Hossain & Poehling 2006). These natural insecticides possess secondary compounds such as terpenoids that protect plants by causing various effects in insects, including behavioral and physiological responses (Tedeschi et al. 2001), but may have adverse effects on natural enemies (Bottrell et al. 1998; Coley et al. 2006). These substances may be more harmful to generalist predators than to pests (Francis et al. 2000; Nishida 2002; Vivan et al. 2002; Coley et al. 2006).

The plant families containing the most promising botanical insecticides are Annonaceae, Asteraceae, Canellaceae, Lamiaceae, Meliaceae, and Rutaceae (Schmutterer 1990; Stein & Klingauf 1990; Mordue & Blackwell 1994; Isman 2006). The use of plant extracts in pest control is increasing due to consumer demand for pesticide-free products (Isman 2006). However, few studies have demonstrated the impact of botanical extracts on natural enemies, thus increasing the need to study their effects on beneficial organisms. The aim of this study was to evaluate the toxicity of aqueous extracts of *Annona squamosa* L. (Magnoliales: Annonaceae), *Azadirachta indica* A. Juss (Sapindales: Meliaceae), *Cymbopogon citriodora* Hook. (Myrtales: Myrtaceae), *Cymbopogon winterianus* Jowitt ex Bor (Poales: Poaceae), *Lippia sidoides* Cham. (Lamiales: Verbenaceae), *Mentha arvensis* L. (Lamiales: Lamiaceae), *Ricinus com-*

munis L. (Malpighiales: Euphorbiaceae), and *Sapindus saponaria* L. (Sapindales: Sapindaceae) on the predator *P. nigrispinus*.

Tests were conducted and insects reared at the Laboratory of Agriculture and Forest Pests of the Department of Forestry, Federal University of Sergipe (UFS), Sergipe, Brazil, under the following conditions: temperature of 25 ± 2 °C, 60 ± 10% relative humidity, and a 12:12 h L:D photoperiod. Adults of *P. nigrispinus* were kept in screened cages measuring 60 × 40 × 40 cm and fed with pupae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae); moistened cotton served as a water source. The egg masses in the cages were collected on the day of oviposition and transferred to Petri dishes (9.0 cm diameter × 1.5 cm height) with moistened cotton. After hatching, the nymphs were retained in those dishes and fed with pupae of *T. molitor* until adulthood. This rearing has been maintained for 1 yr, and new individuals are collected in the field and introduced in the rearing semi-annually.

Aqueous extracts were prepared from plants collected from the experimental plantation at the UFS. We used the leaves of *A. squamosa*, *C. winterianus*, *C. citriodora*, *L. sidoides*, *R. communis*, *S. saponaria*, and *M. arvensis*, and the flowers, leaves, and seeds of *A. indica*. These plants parts were dried at 40 °C for 48 h and ground in an electric mill to obtain a fine powder. Crude extracts were obtained by mixing 10 g of fine powder per 100 mL of distilled water and storing them for 48 h in hermetically sealed containers. The suspensions obtained were filtered using filter paper to obtain a 10% aqueous extract, and were diluted in distilled water to obtain 1, 3, 5, and 7% concentrations. Distilled water was used as negative control and considered to be 0%.

Eggs were collected from the rearing cages within 24 h of oviposition. Nine extracts were assessed at 6 concentrations (0, 1, 3, 5, 7, and 10%) using 5 replicates of 10 eggs, totaling 2,700 eggs. The same tests were conducted with 5th instars and 72-h-old unsexed adults. The eggs were immersed in the extracts or in distilled water (control) and then maintained in air to dry the excess liquid. They were then placed in Petri dishes, and viability (number of nymphs hatched) was evaluated. A similar method was used for nymphs and adults, except that 1 µL of each extract was applied with an automatic pipette to the pronotum of the insects. After application of the aqueous extract, insects were maintained in Petri dishes (9.0 cm diameter × 1.5 cm height) and provided with moistened cotton as a source of water and pupae of *T. molitor* as food. The number of surviving insects was assessed after 72 h.

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The mortality of insects in each test was corrected using Abbott's equation (Abbott 1925). The regression equations for LC50 and/or LC90 and confidence limits of 95% were calculated by probit analysis (Finney 1971) with SAS Proc PROBIT (SAS Institute 1997).

Results showed that extracts of *C. citriodora*, *C. winterianus*, and *M. arvensis* were not toxic to the eggs at the concentrations tested (Table 1). However, the eggs were susceptible to extracts of leaves from *A. squamosa* and *S. saponaria*, and to extracts of seeds and leaves from *A. indica* (Table 1). Those results were expected because the extracts of *A. squamosa* (Sonkamble et al. 2000; Dharmasena et al. 2001; Khaleqzaman & Sultana 2006, Kumar et al. 2010), *S. saponaria* (Santos et al. 2008; Porras & López-Ávila 2009), and *A. indica* (Isman 2006; Tavares et al. 2010) are used for controlling agricultural pests.

Fifth instars tolerated the botanical extracts, making it impossible to prepare dose-response curves. In this study, negative impact on the fifth instars exposed to the extracts was not observed, although their detoxification may affect nymphal duration and reproduction of adults (Tedeschi et al. 2001), which was not evaluated in our study. The mortality of adults (Table 1) was less than 50% with *A. squamosa*, *A. indica* (leaves), *C. citriodora*, *M. arvensis*, and *R. communis* extracts. By comparison, the adults were more susceptible to the extracts of *A. indica* flowers, *C. winterianus*, and *S. saponaria* (Table 1). Thus, the botanical extracts of *A. indica*, *C. winterianus*, and *S. saponaria* have potentially useful insecticidal properties (Schmutterer 1990; Stein & Klingauf 1990; Nawrot et al. 1991; Mordue & Blackwell 1993).

The extracts of *C. citriodora*, *L. sidoides*, and *M. arvensis* did not affect the predator *P. nigrispinus*, but are known to be effective in controlling *Anticarsia gemmatilis* Hübner, *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae) (Chrispeels & Raikhel 1991; Wilson et al. 1997; Tedeschi et al. 2001; Batish et al. 2008; Berthold Vargas et al. 2009; Mota et al. 2012), and other defoliating lepidopteran larvae. These extracts potentially could be used in conjunction with *P. nigrispinus* in integrated pest management programs. However, the extracts of *A. indica*, *A. squamosa*, *C. winterianus*, and *S. saponaria* reduced *P. nigrispinus*' egg viability and adult survival, indicating the need for care when used for pest control to avoid harming non-target insects. This study showed that botanical extracts vary in their potential suitability for use in integrated pest management programs—although some are selective, others can injure biological control organisms.

Summary

The aim of this study was to evaluate the toxicity of aqueous extracts of *Annona squamosa* L. (Magnoliales: Annonaceae), *Azadirachta indica* A. Juss (Sapindales: Meliaceae), *Corymbia citriodora* Hook. (Myrtales: Myrtaceae), *Cymbopogon winterianus* Jowitt ex Bor (Poales: Poaceae), *Lippia sidoides* Cham. (Lamiales: Verbenaceae), *Mentha arvensis* L. (Lamiales: Lamiaceae), *Ricinus communis* L. (Malpighiales: Euphorbiaceae), and *Sapindus saponaria* L. (Sapindales: Sapindaceae) on the developmental stages (eggs, 5th instars, and adults) of a generalist predator *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae). The eggs were dipped in extract concentrations of 0, 1, 3, 5, 7, and 10%, and the nymphs and adults received 1 µL of each extract on the dorsal thorax. The extracts affected hatching, with the extracts of *A. indica* (flowers), *A. squamosa*, and *R. communis* being the most toxic. None of the extracts affected the mortality in the nymphal stage, whereas *S. saponaria*, *A. indica* (flowers), and *L. sidoides* extracts caused relatively high mortality rates in adults. Due to the insecticidal effect of these extracts, they need to be used with care because they affect the life cycle of the predator *P. nigrispinus*.

Key Words: alternative control; natural insecticide; predatory bug

Table 1. LC50 and LC90 of botanical extracts against eggs and adults of *Podisus nigrispinus* (Hemiptera: Pentatomidae).

Botanical extracts	Eggs				Adults					
	LC50 (µL/mL)	LC90 (µL/mL)	χ ²	Slope	P	LC50 (µL/mL)	LC90 (µL/mL)	χ ²	Slope	P
<i>Annona squamosa</i>	4.6 (0.26–0.60)	14.8 (11.9–21.6)	20.60	1.25 ± 0.72	0.29	n/a	n/a	n/a	n/a	n/a
<i>Azadirachta indica</i> (flowers)	32.6 (1.49–8.2)	6,289 (74.2–55,720)	11.00	0.56 ± 0.17	0.97	32.5 (9.4–64.0)	2,468 (40.1–5,252)	10.86	0.68 ± 0.26	0.89
<i>A. indica</i> (green leaves)	102.9 (5.1–3554)	31.3 (1.9–18.6)	13.10	0.60 ± 0.26	0.78	n/a	n/a	n/a	n/a	n/a
<i>A. indica</i> (seeds)	10.1 (0.80–1.6)	2,383 (52.8–5,774)	18.15	0.94 ± 0.97	0.09	n/a	n/a	n/a	n/a	n/a
<i>Corymbia citriodora</i>	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
<i>Cymbopogon winterianus</i>	n/a	n/a	n/a	n/a	n/a	43.0 (20.0–299)	296.1 (45.9–2,991)	16.08	0.70 ± 0.26	0.58
<i>Ricinus communis</i>	34.4 (1.29–6.91)	360.0 (129.1–1,169)	36.03	3.44 ± 1.29	0.05	n/a	n/a	n/a	n/a	n/a
<i>Sapindus saponaria</i>	12.5 (0.98–2.1)	3336 (528.2–5,774)	11.03	1.00 ± 0.30	0.09	62.2 (3.3–33.73)	466.7 (58.3–8,231)	12.96	0.68 ± 0.26	0.79
<i>Mentha arvensis</i>	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

n/a, not applicable

Sumário

O objetivo desse estudo foi avaliar a toxicidade dos extratos aquosos da *Annona squamosa* L. (Magnoliales: Annonaceae), *Azadirachta indica* A. Juss (Sapindales: Meliaceae), *Corymbia citriodora* Hook. (Myrtales: Myrtaceae), *Cymbopogon winterianus* Jowitt ex Bor (Poales: Poaceae), *Lippia sidoides* Cham. (Lamiales: Verbenaceae), *Mentha arvensis* L. (Lamiales: Lamiaceae), *Ricinus communis* L. (Malpighiales: Euphorbiaceae), e *Sapindus saponaria* L. (Sapindales: Sapindaceae) nos estágios de desenvolvimento (ovos, quinto instar e adultos) do predador generalista *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae). Os ovos foram imergidos em extratos com concentrações de 0, 1, 3, 5, 7 e 10%, ninfas e adultos do predador receberam 1 µl de cada extrato na área dorsal. Os extratos de *A. indica* (flores), *A. squamosa* e *R. communis* afetaram a eclosão de ninfas. Nenhum dos extratos avaliados afetou a mortalidade nas ninfas, porém os extratos de *S. saponaria*, *A. indica* (flores) e *L. sidoides* causaram alta mortalidade aos adultos. Apesar desses extratos possuírem ação inseticidas, eles devem ser utilizados com cuidado, pois podem afetar o ciclo de vida do predador *P. nigrispinus*.

Palavras Chave: controle alternativo; inseticidas naturais; percepção do predador

References Cited

- Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265–267.
- Batish DR, Harminder PS, Ravinder KK, Shalinder K. 2008. Eucalyptus essential oil as a natural pesticide. *Forest Ecology and Management* 256: 2166–2174.
- Berthold Vargas LR, Nascimento MJ, Bordin D, Salvador M, Eduard SA, Monteiro de Barros N, Barbieri L, Stirpe F, Carlini CR. 2009. Type 1 ribosome-inactivating proteins—entomotoxic, oxidative and genotoxic action on *Anticarsia gemmatalis* (Hübner) and *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). *Journal of Insect Physiology* 55: 51–58.
- Bottrell DG, Barbosa P, Gould F. 1998. Manipulating natural enemies by plant variety selection and modification: a realistic strategy? *Annual Review of Entomology* 43: 347–367.
- Campiche S, Slooten KB, Ridreau C, Tarradellas J. 2006. Effects of insect growth regulators on the nontarget soil arthropod *Folsomia candida* (Collembola). *Ecotoxicology and Environmental Safety* 63: 216–225.
- Chrispeels MJ, Raikhel NV. 1991. Lectins, lectin genes and their role in plant defense. *Plant Cell* 3: 1–9.
- Coley PD, Bateman ML, Kursar TA. 2006. The effects of plant quality on caterpillar growth and defense against natural enemies. *Oikos* 115: 219–228.
- Dharmasena CMD, Blaney WM, Simmonds MSJ. 2001. Effect of storage on the efficacy of powdered leaves of *Annona squamosa* for the control of *Callosobruchus maculatus* on cowpeas (*Vigna unguiculata*). *Phytoparasitica* 29: 191–196.
- Finney DJ. 1971. *Probit Analysis*. Cambridge University Press, Cambridge, United Kingdom.
- Francis F, Haubruge E, Gaspar C. 2000. Influence of host plants on specialist/generalist aphids and on the development of *Adalia bipunctata* (Coleoptera: Coccinellidae). *European Journal of Entomology* 97: 481–485.
- Hossain MB, Poehling HM. 2006. Non-target effects of three biorationale insecticides on two endolarval parasitoids of *Liriomyza sativae* (Diptera, Agromyzidae). *Journal of Applied Entomology* 130: 360–367.
- Isman MB. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology* 51: 45–66.
- Khalequzzaman M, Sultana S. 2006. Insecticidal activity of *Annona squamosa* L. seed extracts against the red flour beetle, *Tribolium castaneum* (Herbst). *Journal of Biosciences* 14: 107–112.
- Kumar JA, Rekha T, Devi SS, Kannan M, Jaswanth A, Gopal V. 2010. Insecticidal activity of ethanolic extract of leaves of *Annona squamosa*. *Journal of Chemical and Pharmaceutical Research* 2: 177–180.
- Lemos WP, Ramalho FS, Serrão JE, Zanuncio JC. 2003. Effects of diet on development of *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae), a predator of the cotton leafworm. *Journal of Applied Entomology* 127: 389–395.
- Lemos WP, Ramalho FS, Serrão JE, Zanuncio JC. 2005. Morphology of female reproductive tract of the predator *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) fed on different diets. *Brazilian Archives of Biology and Technology* 48: 129–138.
- Matos-Neto FC, Zanuncio JC, Picanço MC, Cruz, I. 2002. Reproductive characteristics of the predator *Podisus nigrispinus* (Heteroptera: Pentatomidae) fed with an insect resistant soybean variety. *Pesquisa Agropecuária Brasileira* 37: 917–924.
- Mordue AJ, Blackwell A. 1993. Azadirachtin: an update. *Journal of Insect Physiology* 39: 903–924.
- Mota ML, Lobo LT, Costa JM, Costa LS, Rocha HA, Rocha e Silva LF, Pohlit AM, Neto VF. 2012. In vitro and in vivo antimalarial activity of essential oils and chemical components from three medicinal plants found in north-eastern Brazil. *Planta Medica* 78: 658–64.
- Nawrot J, Koul O, Isman MB, Harmatha J. 1991. Naturally occurring anti-feedants—effects on 2 polyphagous lepidopterans. *Journal of Applied Entomology* 112: 194–201.
- Nishida R. 2002. Sequestration of defensive substances from plants by Lepidoptera. *Annual Review of Entomology* 47: 57–92.
- Oliveira JEM, Torres JB, Moreira A, Zanuncio JC. 2002. Biologia de *Podisus nigrispinus* predando lagartas de *Alabama argilacea* em campo. *Pesquisa Agropecuária Brasileira* 37: 7–14.
- Porras MF, López-Avila A. 2009. Effect of extracts from *Sapindus saponaria* on the glasshouse whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). *Revista Colombiana de Entomología* 35: 7–11.
- Rocha LCD, Carvalho GA, Moura AP, Cosme LV, Vilela FZ. 2006. Toxicidade de produtos fitossanitários utilizados na cultura do crisântemo para ovos e ninfas de *Orius insidiosus* (Say) (Hemiptera: Anthracoridae). *Neotropical Entomology* 35: 83–92.
- Santos WL, Freire MGM, Bogorni PC, Vendramim JD, Macedo MLR. 2008. Effect of the aqueous extracts of the seeds of *Talisia esculenta* and *Sapindus saponaria* on fall armyworm. *Brazilian Archives of Biology and Technology* 51: 373–383.
- SAS Institute. 1997. *User's Guide: Statistics*. SAS Institute, Cary, North Carolina, USA.
- Schmutterer H. 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annual Review of Entomology* 35: 271–297.
- Silva CAD, Zanuncio TV, Cunha BG, Castro AA, Canevari GC, Serrão JE, Zanuncio JC. 2009. Development and survival of nymphs of *Podisus nigrispinus* (Heteroptera: Pentatomidae) fed with caterpillars of *Chlosyne lacinia saundersii* (Lepidoptera: Nymphalidae). *Brazilian Archives of Biology and Technology* 52: 105–109.
- Silva RA, Carvalho GA, Carvalho CF, Reis PR, Pereira AMAR, Cosme LV. 2005. Toxicidade de produtos fitossanitários utilizados na cultura do caféiro a larvas de *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) e efeitos sobre as fases subsequentes do desenvolvimento do predador. *Neotropical Entomology* 34: 951–959.
- Sonkamble MM, Dhanorkar BK, Munde AT, Sonkamble AM. 2000. Efficacy of indigenous plant extracts against *Helicoverpa armigera* (Hübner) and *Spodoptera litura* (Fabricius) under laboratory conditions. *Journal of Soils and Crops* 10: 236–239.
- Stein U, Klingauf F. 1990. Insecticidal effect of plant extracts from tropical and subtropical species. Traditional methods are good as long as they are effective. *Journal of Applied Entomology* 110: 160–166.
- Tavares WS, Costa MA, Cruz I, Silveira RD, Serrão JE, Zanuncio JC. 2010. Selective effects of natural and synthetic insecticides on mortality of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and its predator *Eriopis connexa* (Coleoptera: Coccinellidae). *Journal of Environmental Science and Health, Part B* 45: 557–561.
- Tedeschi R, Alma A, Tavella M. 2001. Side-effects of three neem (*Azadirachta indica* A. Juss.) products on the predator *Macrolophus caliginosus* Wagner (Heteroptera: Miridae). *Journal of Applied Entomology* 125: 397–402.
- Torres JB, Ruberson JR. 2004. Toxicity of thiamethoxam and imidacloprid to *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) nymphs associated to aphid and whitefly control in cotton. *Neotropical Entomology* 33: 99–106.
- Torres JB, Zanuncio JC, Oliveira HN. 1998. Nymphal development and adult reproduction of the stinkbug predator *Podisus nigrispinus* (Heteroptera: Pentatomidae) under fluctuating temperatures. *Journal of Applied Entomology* 122: 509–514.
- Torres JB, Silva-Torres CSA, Barros R. 2003. Relative effect of the insecticide thiamethoxam on the predator *Podisus nigrispinus* and the tobacco

- whitefly *Bemisia tabaci* in nectaried and nectariless cotton. *Pest Management Science* 59: 315–323.
- Torres JB, Barros EM, Coelho RR, Pimentel RMM. 2010. Zoophytophagous pentatomids feeding on plants and implications for biological control. *Arthropod–Plant Interactions* 4: 219–227.
- Vivan LM, Torres JB, Barros R, Veiga AFSL. 2002. Tasa de crecimiento poblacional del chinche depredador *Podisus nigrispinus* (Heteroptera: Pentatomidae) y de la presa *Tuta absoluta* (Lepidoptera: Gelechiidae) en invernadero. *Revista de Biología Tropical* 50: 145–153.
- Vivan LM, Torres JB, Veiga AFSL. 2003. Development and reproduction of a predatory stinkbug, *Podisus nigrispinus*, in relation to two different prey types and environmental conditions. *BioControl* 48: 155–168.
- Wiesbrook ML. 2004. Natural indeed: Are natural insecticides safer and better than conventional insecticides? *Illinois Pesticide Review* 17: 1–8.
- Wilson CL, Solar JM, Ghaouth AE, Winiewski ME. 1997. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. *Plant Disease* 81: 204–210.