Biological Control 57 (2011) 75–78

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Effects of *Melia azedarach* extract on *Cotesia ayerza*, parasitoid of the alfalfa defoliator *Colias lesbia*

María T. Defagó [a], Analía Dumón [a], Delia S. Avalos [b], Sara M Palacios [c], Graciela Valladares [a,*]

[a] Centro de Investigaciones Entomológicas de Córdoba, Facultad de Cs. Ex. Fs. y Nat. U.N.C., Av. V. Sarsfield 1616, 5000 Córdoba, Argentina
[b] Cátedra de Zoología Agrícola, Facultad de Ciencias Agropecuarias, U.N.C., Av. Valparaíso s/n, Ciudad Universitaria, 5000 Córdoba, Argentina
[c] Laboratorio de Química Fina y Productos Naturales, Universidad Católica de Córdoba, Camino a Alta Gracia Km 10, 5000 Córdoba, Argentina

A R T I C L E I N F O

Article history:
Received 25 November 2009
Accepted 5 January 2011
Available online 19 January 2011

Keywords:
Cotesia ayerza
Melia azedarach
Botanical pesticides
Colias lesbia
Pest control

A B S T R A C T

Extracts from the fruit of *Melia azedarach* L. (Meliaceae) have shown antifeedant and toxic effects on several insect species, including various pests, but the action of these compounds on natural enemies such as predators and parasitoids is less understood. The purpose of this work was to assess extract effects on *Cotesia ayerza* (Brèthes) (Hymenoptera: Braconidae), a parasitoid of the alfalfa defoliator *Colias lesbia* Fab. (Lepidoptera: Pieridae). The extract was first tested on butterfly larvae, in order to determine sublethal concentrations, and thus enable the evaluation of extract effects on parasitoid development from treated hosts. Direct effects of the extract through ingestion or contact (topical application) on adult wasp survival were also evaluated. An extract concentration of 0.2% was selected for parasitoid tests, since it allowed butterfly larvae to survive and pupate at normal rates, while inducing sublethal effects manifested as lower body weight. The indirect effects of the extract, through parasitoid development on treated hosts, were seen only in a female-biased sex ratio, without significant effects on either cocoon formation time, number of cocoons or adult parasitoids emerged. Adult wasps suffered increased mortality when their food was treated with extract, but were unaffected by topical application. These results suggest that *M. azedarach* extracts could represent an interesting tool for integrated pest management of *C. lesbia* populations.

1. Introduction

Plants synthesize secondary metabolites or allelochemicals that have toxic and/or feeding deterrent activity in insects, and thereby contribute to the regulation of their populations (Mareggiani, 2001). Several limonoids with these characteristics have been isolated from various representatives of Meliaceae (Kraus et al., 1986; Wheeler and Isman, 2001). The best known is azadirachtin, considered the most important active ingredient of the neem tree (*Azadirachta indica* A. Juss) (Islam, 1986; Champagne et al., 1989; Ascher, 1993; Breuer and De Loof, 2000). Limonoids were obtained from seeds of another Meliaceae, *Melia azedarach* L., which produce effects similar to those reported for neem (Kraus et al., 1986), and so this is also considered a promising source of natural pesticides (Palacios et al., 1993; Ascher et al., 1995; Defagó et al., 2006). Antifeedant and/or toxic effects of raw extracts or active principles of *M. azedarach* have been reported for a variety of insects, mainly Lepidoptera, Coleoptera, Diptera and Heteroptera (Isman, 1993; Dilawari et al., 1994; Valladares et al., 1997; Serra et al., 1998; Carpinella et al., 2003; Defagó et al., 2009).

Colias lesbia Fab. (Lepidoptera: Pieridae) is outstanding among the economically significant Lepidoptera in alfalfa crops in Argentina, as its larvae can cause losses equivalent to one cut of alfalfa per year (Imwinkelried et al., 1990). Extracts of *M. azedarach* have significantly reduced larval feeding in this species (Valladares et al., 2003; Defagó et al., 2009). *C. lesbia* has a diverse parasitoid assemblage (Avalos, 2007) and so, within a framework of integrated pest management (IPM), it would be useful to know the possible effects of the plant extract on these agents. Information on interactions between *M. azedarach* extracts and natural enemies of target pest insects is currently very scarce (Banchio et al., 2003; Charleston et al., 2005; Charleston et al., 2006).

Within this framework, the present paper deals with the parasitoid most frequently affecting *C. lesbia* in alfalfa fields of Argentina: *Cotesia ayerza* Brèthes (Hymenoptera: Braconidae) (Avalos, 2007). This is a gregarious, koinobiont endoparasitoid, developing within a host while the latter completes its larval stage, then emerging and knitting a cocoon to pupate on the host surface. Here, we first compare the effects of various *M. azedarach* fruit extract concentrations on *C. lesbia* development and survival, and

* Corresponding author.
E-mail address: gvalladares@efn.uncor.edu (G. Valladares).

1049-9644/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2011.01.002

then evaluate whether sublethal concentrations for this pest indirectly affect the fitness of its parasitoid *C. ayerza.* Moreover, we analyze direct effects of this extract – through contact and ingestion – on adult parasitoid survival.

2. Materials and methods

2.1. Insect breeding

2.1.1. Lepidoptera host

C. lesbia larvae and eggs were collected in spring-summer 2007–2008 from alfalfa crops in Córdoba University agricultural field station (Capilla de los Remedios, to 25 km of Córdoba city (31° 29′ S and 64° 00′ O). Newly emerged *C. lesbia* caterpillars were placed in plastic cups (9 cm high, 8 cm diameter) with a gauze cover, and fed on fresh alfalfa sprigs from the field station, renewed every 48 h. Adults were placed in entomological cages (wooden frames, glass and voile walls, 30 × 30 × 30 cm), provided with a sugar solution and alfalfa sprigs as oviposition substrate. Leaves with eggs were placed in Petri dishes until eggs hatched (adapted from Bahena et al., 1998). Third larval instars of *C. lesbia* were used for the tests.

2.1.2. Parasitoids

A parasitoid colony was started in the laboratory from parasitized *C. lesbia* larvae collected in the field. The emerged adult wasps were conditioned in entomological cages (see Section 2.1.1), fed a 10% sugar solution (Geervliet et al., 1998) and offered host larvae for oviposition. *C. ayerza* adults were used in the tests 24 h after emergence.

All tests were carried out under controlled humidity (65 ± 5%), temperature (26 ± 2 °C) and photoperiod (12:12 h) conditions.

2.2. Botanical Extracts

Ripe fruits of *M. azedarach* were collected from trees in Córdoba (Argentina) in October 2007. A voucher specimen has been deposited at the Botanical Museum of Córdoba (CORD 229, Córdoba, Argentina). Air-dried plant material (20–26 °C), was crushed in a bench miller, Soxhlet extracted with ethanol and concentrated under vacuum, to yield 28.82 g% fruit extract (Carpinella et al., 2003). An exact amount of the fruit extract was weighted and diluted in distilled (10 ml) water to reach 5, 2, 0.50, 0.20 or 0.02 g% concentration. To improve adhesion to the leaf surface, 0.07 ml of the surfactant "Tween 20" was added to the control and to each extract concentration.

2.3. Bioassays

2.3.1. Determination of sublethal dose in C. lesbia

To determine sublethal doses, methodology was adapted from Matter et al. (2002). One larva of *C. lesbia* (third instar) was placed per Petri dish and was given alfalfa leaves painted on both faces with concentrations lower than 1% extract or with water (control), until survival rates were similar to those of control larvae (see Fig. 1) (Carpinella et al., 2003; Valladares et al., 2003). For the different treatments, body weight, mortality and amount of food ingested (% leaf area, visually estimated as in Defagó et al., 2009) were recorded every 48 h. Ten replicates were performed for each treatment. For comparisons among treatments, cumulative prepupal mortality, body weight increase (at the last date with available larvae in all treatments) and % leaf area consumed were considered. Data were analyzed with ANOVA or Kruskal–Wallis depending on whether data normality and homocedasticity requirements were met.

2.3.2. Indirect effects of the extract on the development of C. ayerza

In each entomological cage, four larvae of *C. lesbia* were placed on alfalfa leaves treated with the extract in the selected sublethal dose, or with water (control). After 48 h, four ♀♀ and two ♂♂ of *C. ayerza* were introduced in the cages containing the lepidoptera larvae. The food of the parasitoids (water and honey) and that of the host larvae were renewed every 48 h until pupation of *C. lesbia* or emergence of the parasitoids (nine repetitions were performed). Cocoon formation time, number of cocoons obtained, and number of parasitoids emerged, were recorded. Male and female adult wasps thus reared were identified and counted in order to establish sex ratio. Wasp hind tibial length was measured to provide an indicator of adult fitness (Charleston et al., 2005).

Variations in cocoon formation time were analyzed with the Mann–Whitney test. Differences between treatments regarding number of cocoons, number of parasitoids and tibial length of ♀♀ and ♂♂, were analyzed through the "*t*" test or the Wilcoxon. Parasitoid sex frequency was analyzed within treatments by means of the X^2 test.

2.3.3. Assessing the direct effect of extract on adult parasitoids

2.3.3.1. Ingestion. Five adult specimens of *C. ayerza,* randomly selected from the laboratory colony, were placed in a cage (as in Section 2.1) and offered a food solution consisting of sugar (1 ml), water (4 ml) and *M. azedarach* extract (5 ml), with final extract concentrations of 2%, and 5%. A similar control group was fed with water (9 ml) and sugar (1 ml). Five replicates were made for each extract concentration according to Matter et al. (2002) and mortality was recorded. Mortality rates were compared among treatments by means of ANOVA or Kruskal–Wallis and regression analysis.

2.3.3.2. Topical application. In order to evaluate contact effects, *C. ayerza* wasps randomly selected from the laboratory colony were isolated in entomological cages (as in Section 2.1), sprayed either with 1 ml extract at 2% or 5% or with water (control), and fed with a sugar solution. Four replicates of five individuals were made for each extract concentration and mortality was recorded every 72 h. Mortality rates were compared among treatments by means of ANOVA or Kruskal–Wallis.

3. Results

3.1. Determination of sublethal dose in C. lesbia

Feeding on extract-treated food resulted in dose-dependent larval mortality for *C. lesbia*, (ANOVA: $F_{0.05\ (3,8)} = 19.94$; $P = 0.001$). All

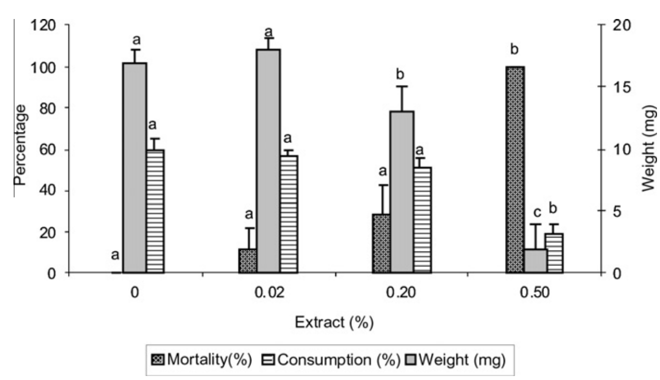

Fig. 1. Effects of different doses of *M. azedarach* extract on aspects of *C. lesbia* larva biology. Mean (±SE) cummulative prepupal mortality, % leaf area consumed and weight gain (after 15 days of treatment), different letters indicate significant differences (Tukey < 0.05).

larvae receiving the highest concentration (0.5%) died within a fortnight without entering the pupal stage. In contrast, those fed with concentrations of 0.2% and 0.02% did so in percentages close to those of the controls.

Larvae receiving food treated with 0.5% extract ate less than those fed with lower extract doses (0.02% or 0.2%), whose consumption was in turn similar to that of larvae facing untreated food (Fig. 1). This trend was statistically significant (ANOVA: $F_{0.05\ (3,29)} = 11.84$; $P < 0.001$).

The weight of *C. lesbia* larvae fed with extract concentrations of 0.5% and 0.2% was significantly lower (ANOVA: $F_{0.05\ (3,26)} = 35.97$; $P = 0.001$) than that recorded for 0.02% and the control (Fig. 1). Consequently, the 0.2% extract dose, affecting host weight but not its survival, was selected for parasitoid assays.

3.2. Indirect effects of the extract on the development of C. ayerza

Parasitoids developing on *C. lesbia* larvae were not affected by their hosts receiving food treated with 0.2% *M. azedarach* extract. No significant differences between treatments were found regarding the number of cocoons yielded (treated: 44 ± 8.55, untreated: 43 ± 6.8, $t = -0.06$, $P = 0.956$), the days required for cocoon formation (treated: 15.25 ± 1.05; untreated: 17.33 ± 1.28, $W = 55$, $P = 0.25$), the number of adult parasitoids reared from those cocoons (treated: 42 ± 8.54, untreated: 42 ± 6.39, $t = -0.08$, $P = 0.937$). The presence of the extract in the food of the hosts did not affect the tibial length of adults (♀♀ treated: 0.69 ± 0.01, untreated: 0.68 ± 0.01, $t = -0.82$, $P = 0.41$; ♂♂ treated: 0.63 ± 0.01, untreated: 0.63 ± 0.01, $t = 0.14$, $P = 0.88$).

The sex ratio of the parasitoids emerged from control larvae was 1♀:1♂ ($X^2 = 0.4$, df = 1, $P = 0.52$), while in those emerging from extract-fed larvae a 1♀:2♂ ratio was recorded ($X^2 = 48.42$, $P \leqslant 0.001$) (Fig. 2).

3.3. Assessing the direct effect of extract on adult parasitoids

3.3.1. Ingestion effects

The ingestion of *M. azedarach* fruit extract impaired the survival of *C. ayerza* (Fig. 3). In the extract-fed parasitoids, mortality exceeded by 7–8 times that seen in controls on the fourth experiment day (ANOVA: $F_{0.05\ (2,11)} = 23.24$; $P = 0.0001$), this activity was strongly dose-dependent ($r^2 = 0.75$; $P = 0.0001$). Both treatments with extract reached 100% mortality on the fifth day, while controls survived 24 h more.

3.3.2. Effects of topical application

Wasp survival was not affected by spraying *M. azedarach* extract directly on adults (Table 1, Kruskal–Wallis $P > 0.05$ in all cases).

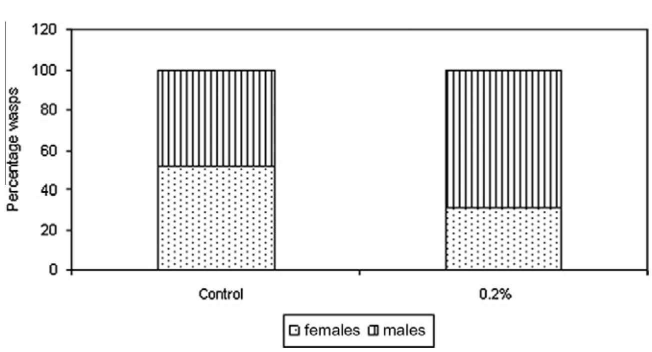

Fig. 2. Sex ratio (%) of *C. ayerza* emerged from larvae of *C. lesbia* fed with sublethal doses of *M. azedarach* extract.

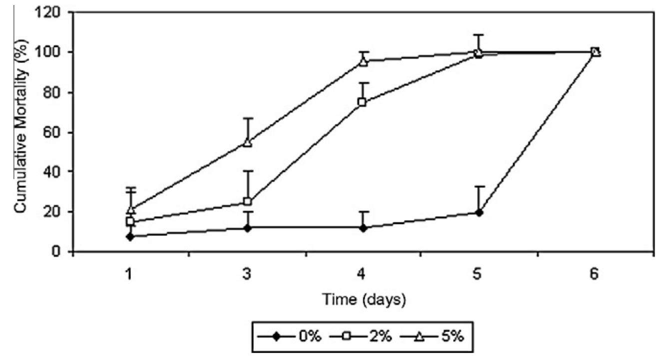

Fig. 3. Mean (±SE) cumulative mortality of *C. ayerza* fed with different doses of *M. azedarach* extract. (Each point represents the mean of four replicates of five initial individuals each).

Table 1
Accumulated mortality rates (± SE) resulting from topical application of *Melia azedarach* fruit extract on *Cotesia ayerza* adults.

Treatment (%)	Days 1	3	5
0	20 (± 7.3)	46 (± 8.4)	100 (± 0.0)
2	43 (± 9.7)	76 (± 6.1)	97 (± 3.3)
5	35 (± 8.6)	66 (± 8.8)	100 (± 0.0)

Kruskal-Wallis $P > 0.05$ in all cases.

4. Discussion

Consideration of direct and indirect effects of botanical pesticides on higher trophic levels, as represented by parasitoids, is a necessary step in order to evaluate and understand their bioactivity and their scope as tools for pest control. The fruit extract of *M. azedarach*, at a dose of 0.5%, significantly reduced food consumption and body weight of *C. lesbia* larvae. These results support and expand previous reports, in which antifeedant effects were observed on this insect using higher extract concentrations than those tested here (Palacios et al., 1993; Carpinella et al., 2003; Valladares et al., 2003). Also at 0.5% concentration, pupation was completely inhibited and 100% mortality was reached, as observed with other lepidopteran species exposed to *M. azedarach* extracts (e.g. Schmidt et al., 1997; Breuer and De Loof, 1998; De Brito et al., 2004; Torres et al., 2006; Rossetti et al., 2008). At the other extreme, no noticeable effects were shown by *C. lesbia* larvae receiving food treated with 0.02% extract.

For the purposes of this study, an extract concentration of 0.2% appeared most adequate, allowing butterfly larvae to survive and pupate at normal rates, while inducing sublethal effects manifested as lower body weight. Smaller larvae might be less efficient in their capacity to encapsulate parasitoids (Perera et al., 2000), which would then benefit from the extract effects. On the other hand, parasitoid size is frequently correlated to host size (Harvey et al., 2000). Moreover, extracts in the host body could be toxic to the parasitoids or otherwise impair their development (Price and Schuster, 1991; Lowery and Isman, 1995), or even attract them, facilitating host location (Charleston et al., 2006). Studies with neem extracts have recorded null or negative effects on parasitoids (e.g. Perera et al., 2000; Viñuela et al., 2000; Akol et al., 2003; Boeke et al., 2003; Mitchell et al., 2004), although some authors have queried this harmlessness (Lowery and Isman, 1995). In the present study, parasitoids reared from *C. lesbia* treated with *M. azedarach* extracts did not differ from those reared from untreated larvae, either in numbers, survival, development time or adult size. Only the sex ratio was altered, in favor of males, on treated hosts. A similar trend, which might negatively affect parasitoid numerical response, was observed in another species of *Cotesia* when reared from hosts treated with *M. azedarach* leaf extracts, with the additional effect of males being smaller (Charleston et al., 2005).

Direct effects of the extract on adult *C. ayerza* varied depending on application method. Continued exposure to treated food resulted in increased, dose-dependent mortality. This result contrasts with the lack of effects recorded in similar tests on the Hymenoptera parasitoids *Hyposoter ebeninus* and *Cotesia plutella* (Matter et al., 2002; Charleston et al., 2005). On the other hand, topical application of the extract did not affect *C. ayerz*a adults, as observed (Chiasson et al., 2004) for neem oil on *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae).

The present results suggest interesting possibilities for *M. azedarach* extracts in pest management, within a framework of botanical pesticide in combination with biological control (Charleston et al., 2006). Compounds that negatively affect fitness of the target insect while having minimal effects on its natural enemies, could be considered as IPM compatible tools within management pest programs and deserve further study in this context.

Acknowledgments

We thank the Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba for the subsidy for this research. Special thanks to Joss Heywood for reviewing the manuscript translation.

References

Akol, A.M., Njagi, P.G.N., Sithanantham, S., Mueke, J.M., 2003. Effects of two neem insecticide formulations on the attractiveness, acceptability and suitability of diamondback moth larvae to the parasitoid, *Diadegma mollipla* (Holmgren) (Hym., Ichneumonidae). Journal of Applied Entomology 127, 325–331.

Ascher, K.R.S., 1993. Nonconventional insecticidal effects of pesticides available from the neem tree *Azadirachta indica*. Archives of Insect Biochemistry and Physiology 22, 433–449.

Ascher, K.R.S., Schmutterer, H.S., Zebits, C.P.W., Naqvi, S.N.H., 1995. The Persian lilac or chinaberry tree: *Melia azedarach*. In: Schmutterer, H. (Ed.), The Neem tree *Azadirachta indica* A, Juss. and other Meliaceous plants. VCH, Germany, pp. 605–633.

Avalos, S., 2007. Tramas tróficas de Lepidoptera defoliadoras en alfalfa y soja y sus parasitoides. Tesis Doctoral. Universidad Nacional de Córdoba, Argentina. 167 pp.

Bahena, F., Gonzáles, M., Viñuela, E., Del Estal, P., 1998. Establecimiento de la especie huésped óptima para la cría en laboratorio del parasitoide de noctuidos *Hyposoter didymator* (Thunberg). Boletín de Sanidad Vegetal-Plagas 24, 465–472.

Banchio, E., Valladares, G., Defagó, M.T., Palacios, S., Carpinella, M.C., 2003. Effect of *Melia azedarach* (Meliaceae) fruit extracts on the leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae): Assessment in laboratory and field experiments. Annals of Applied Biology 143, 187–193.

Boeke, S.J., Sinzogan, A.A.C., de Almeida, R.P., de Boer, P.W.M., Jeong, G., Kossou, D.K., Van Loon, J.J.A., 2003. Side-effects of cowpea treatment with botanical insecticides on two parasitoids of *Callosobruchus maculatus*. Entomologia Experimentalis et Applicata 108, 43–51.

Breuer, M., De Loof, A., 1998. Meliaceous plant preparations as potential insecticides for control of the oak processionary, *Thaumetopoea processionea* (L.) (Lepidoptera: Thaumetopoeidae). Mededelingen Faculteit Landbouwwetenschappen Universiteit Gent 63, 529–536.

Breuer, M., De Loof, A., 2000. Efficacy of an enriched *Melia azedarach* L. fruit extract for insect control. In: Kleeberg, H., Zebits, W. (Eds.), Practice oriented results on use and production of neem ingredients and pheromones VIII. Druck and Graphic, Giessen, pp. 173–183.

De Brito, C.H., Mezzomo, J.A., Batista, J.L., Barbosa Lima, M.S., Murata, A.T., 2004. Bioatividade de extratos vegetais aquosos sobre *Spodoptera frugiperda* em condições de laboratorio. Manejo Integrado de Plagas y Agroecología 71, 41–45.

Carpinella, M.C., Defagó, M.T., Valladares, G., Palacios, S., 2003. Antifeedant and insecticide properties of a limonoid from *Melia azedarach* (Meliaceae) with potential use for pest management. Journal of Agricultural and Food Chemistry 51, 369–374.

Champagne, D.E., Isman, M.B., Towers, G.H.N., 1989. Insecticidal activity of phytochemicals and extracts of the Meliaceae. In: Arnason, J.T., Philogéne, R.J.R., Morand, P. (Eds.), Insecticides of Plant Origin, ACS Symposium Series 387. American Chemical Society, Washington, pp. 95–109.

Charleston, D.S., Kfir, R., Dicke, M., Vet, L.E.M., 2005. Impact of botanical pesticides derived from *Melia azedarach* and *Azadirachta indica* on the biology of two parasitoid species of diamondback moth. Biological Control 33, 131–142.

Charleston, D.S., Kfir, R., Dicke, M., Vet, L.E.M., 2006. Impact of botanical extracts derived from *Melia azedarach* and *Azadirachta indica* on populations of *Plutella xylostella* and its natural enemies: a field test of laboratory findings. Biological Control 39, 105–114.

Chiasson, H., Vincent, C., Bostanian, N.J., 2004. Insecticidal properties of a *Chenopodium*-based botanical. Journal of Economic Entomology 97, 1378–1383.

Defagó, M., Valladares, G., Banchio, E., Carpinella, C., Palacios, S., 2006. Insecticide and antifeedant activity of different plant parts of *Melia azedarach* (Meliaceae) on *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae). Fitoterapia 77, 500–505.

Defagó, M.T., Mangeaud, A., Benesovsky, V., Trillo, C., Carpinella, C., Palacios, S., Valladares, G., 2009. *Melia azedarach* extracts: a potential tool for insect pest management. In: Singh, V.K., Govil, J.N. (Eds.), Phytopharmacology and Therapeutic Values V. Studium Press LLC, USA, pp. 17–33.

Dilawari, V.K., Singh, K., Dhaliwal, G.S., 1994. Effects of *Melia azedarach* L. on oviposition and feeding of *Plutella xylostella* L. Insect Science and its Application 15, 203–205.

Geervliet, J.B., Ariëns, S., Dicke, M., Vet, L.E., 1998. Long-distance assessment of patch profitability through volatile infochemicals by the parasitoids *Cotesia glomerata* and *C. Rubecula* (Hymenoptera: Braconidae). Biological Control 11, 113–121.

Imwinkelried, J.M., Salto, C.E., Frana, J.E., Berra, I.A., Quaino, O.R., 1990. Entomofauna presente en un alfalfar del centro oeste de Santa Fe. Publicación Técnica INTA Estación Experimental Rafaela 49, 1–18.

Harvey, J.A., Kadash, K., Strand, M.R., 2000. Differences in larval feeding behaviour correlate with altered developmental strategies in two parasitic wasps: implications for the size-fitness hypothesis. Oikos 88, 621–629.

Islam, B.N., 1986. Use of some extracts from Meliaceae and Annonaceae for control of rice hispa, *Dicladispa armigera,* and the pulse beetle, *Callosobruchus chinensis*. In: Schmutterer, H., Ascher, K.S. (Eds.), Natural Pesticides from the Neem Tree and Other Tropical Trees. Nairobi, Kenya, pp. 217–242.

Isman, B.M., 1993. Growth inhibitory and antifeedant effects of azadirachtin on six noctuids of regional economic importance. Pesticide Science 38, 57–63.

Kraus, W., Baumann, S., Bokel, M., Keller, U., Klenk, A., Klingele, M., Pöhnl, H., Schwinger, M., 1986. Control of insect feeding and development by constituents of *Melia azedarach* and *Azadirachta indica*. In: Schmutterer, H., Ascher, K.S. (Eds.), Natural Pesticides from the Neem Tree and Other Tropical Trees. Nairobi, Kenya, pp. 111–125.

Lowery, D.T., Isman, M.B., 1995. Toxicity of neem tree to natural enemies of aphids. Phytoparasitica 23, 297–306.

Mareggiani, G., 2001. Manejo de insectos plaga mediante sustancias semioquímicas de origen vegetal. Manejo Integrado de Plagas y Agroecologia 60, 22–30.

Matter, M.M., Gesrah, M.A., Ahmed, A.A.I., Farag, N.A., 2002. Impact of neem and chinaberry fruit extracts on the pest/parasitoid (*Pieris rapae*/*Hyposoter ebeninus*) interactions. Journal of Pest Science 75, 13–18.

Mitchell, P., Gupta, R., Singh, A.K., Kumar, P., 2004. Behavioral and developmental effect of Neem extract on *Clavigralla* (Hemiptera: Coreidae) and its egg parasitoid, *Gryon fulviventre* (Hymenoptera: Scelionidae). Journal of Economic Entomolology 97, 916–923.

Palacios, S., Valladares, G., Ferreira, D., 1993. Preliminary results in the searching of an insecticide from *Melia azedarach* extracts. In: Kleeberg, H. (Ed.), Practice Oriented Results on Use and Production of Neem Ingredients and Pheromones. Proc. of the First Workshop, Wetzlar. Druck and Graphic, Giessen, pp. 91–105.

Perera, D.R., Armstrong, G., Senanayake, N., 2000. Effect of antifeedants on the diamondback moth (*Plutella xylostella*) and its parasitoid *Cotesia plutellae*. Pest Management Science 56, 486–490.

Price, J.F., Schuster, D.J., 1991. Effects of natural and synthetic insecticides on sweet potato whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) and its hymenopterous parasitoids. The Florida Entomologist 74, 60–68.

Rossetti, M.R., Defagó, M.T., Carpinella, M.C., Palacios, S.M., Valladares, G., 2008. Actividad biológica de extractos de *Melia azedarach* sobre larvas de *Spodoptera eridania* (Lepidoptera:Noctuidae). Revista de la Sociedad Entomológica Argentina 67 (1–2), 115–125.

Schmidt, G.H., Ahmed, A.I., Breuer, M., 1997. Effect of *Melia azedarach* extract on larval development and reproduction parameters of *Spodoptera littoralis* (B.) and *Agrotis ipsilon* (H.) (Lep. Noctuidea). Journal Pest Science 70, 4–12.

Serra, A.J., Badia, A.S., Viladot, M.R., 1998. Caracterización de la actividad alimentaria de extractos de fruto y semilla de *Melia azedarech* L. y de *Azadirachta indica* A. Juss. sobre larvas de Lepidoptera *Sesamia nonagrioides* Lef. Boletín de Sanidad Vegetal-Plagas 24, 1019–1032.

Torres, A.L., Boiça Júnior, A.L., Medeiros, C.A., Barros, R., 2006. Effeito de extratos aquosos de *Azadirachta indica*, *Melia azedarach* e *Aspidosperma pyrifolium* no desenvolvimento e oviposição de *Plutella xylostella*. Fitossanidade 65, 447–457.

Valladares, G., Defagó, M.T., Palacios, S., Carpinella, M.C., 1997. Laboratory evaluation of *Melia azedarach* (Meliaceae) extracts against the elm leaf beetle (Coleoptera: Chrysomelidae). Journal of Economic Entomology 90, 747–750.

Valladares, G., Garbin, L., Defagó, M.T., Carpinella, C., Palacios, S., 2003. Actividad antialimentaria e insecticida de un extracto de hojas senescentes de *Melia azedarach* (Meliaceae). Revista de la Sociedad Entomológica Argentina 62, 53–61.

Viñuela, E., Adán, A., Smaggne, G., Gonzáles, M., Medina, M.P., Budia, F., Vogt, H., Del Estal, P., 2000. Laboratory effects of ingestion of azadirachtin by two pests (*Ceratitis capitata* and *Spodoptera exigua*) and three natural enemies (*Chrysoperla carnea, Opius concolor* and *Podisus maculiventris*). Biocontrol Science and Technology 10, 165–177.

Wheeler, D.A., Isman, M.B., 2001. Antifeedant and toxic activity of *Trichillia americana* extract against the larvae of *Spodoptera litura*. Entomologia Experimentalis et Applicata. 98, 9–16.