Effect of *Flourensia oolepis* Blake (chilca) Extract on Adults of *Helicoverpa gelotopoeon* Dyar (Lepidoptera: Noctuidae) and its Parasitoid *Archytas* sp. (Diptera: Tachinidae)

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ABSTRACT Indiscriminate use of synthetic insecticides, commonly-used tools for pest control, has been shown to cause irreversible environmental damage. Botanical compounds currently represent ecologically acceptable alternatives. The active ingredient of *Flourensia oolepis* (Asteraceae) has been effectively used against different species of insect pests. The aim of this research was to study the effect of the crude extract of *F. oolepis* on the reproduction of *Helicoverpa gelotopoeon* and the survival of parasitoid adults *Archytas* sp. (Tachinidae). To measure the effect of the extract on oviposition, choice tests were conducted, using different extract concentrations (1% to 5%) and acetone (control), and the inhibition rate was calculated. The intake of extract was analyzed by no-choice tests, and adult longevity, oviposition period, fertility, fecundity of *H. gelotopoeon* and survival of the parasitoid *Archytas* sp. was determined. In choice tests, the 2.5% dose of the extract produced a marked oviposition deterrent effect. In non-choice tests in adults, extracts did not affect longevity, but did affect fecundity and fertility when females ingested a 5% dose throughout the experimental period. The intake of extract significantly decreased parasitoid survival.

KEY WORDS : Botanical insecticide, *Flourensia oolepis*, *Helicoverpa gelotopoeon*, parasitoids, pest, reproduction

INTRODUCTION

The South American bollworm moth, *Helicoverpa gelotopoeon* Dyar (Noctuidae), is know for causing extensive damage to multiple crops in the province of Córdoba-Argentina (Fichetti *et al.*, 2009; Iannone, 2011). They consume leaves, tender stems, petioles and flowers (Igarzábal *et al.*, 2011), chickpea pods (Fichetti *et al.*, 2009), soya beans (Flores, 2009) and cotton bolls (Casuso *et al.*, 2012). This species is endemic to Argentina, Chile and Uruguay (Cork and Lobos, 2003). These noctuid populations are regulated by several agents of natural mortality including various species of Diptera: Tachinidae (Stireman *et al.*, 2006), particularly the genera *Gonia* Meigen, *Eucelatoria* Townsend and *Archytas* Jaennicke (Vergara de

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Sánchez and Raven, 1990; Cave 1993).

In recent decades, given the high incidence of pests on different crop systems, chemical insecticides have been used indiscriminately, leading to changes in the ecological balance and generating resistance (Trujillo Ruiz *et al.,* 2008), soil and water pollution, and toxic effects on beneficial organisms, vertebrates and humans (Devine *et al*., 2008). Alternatives methods particularly botanical insecticides that can be included in management programs have begun to be explored (Alonso, 1999; Goudegnon *et al*., 2000).

Plants possess various chemicals (allelochemicals) such as terpenoids, alkaloids, flavonoids, etc. to defend themselves against insects and other threats (Schoonhoven *et al*., 2008; Gil Clavijo *et al*., 2010). They may act as insecticides, repellents, antifeedants or sterilants (Valladares *et al*., 2003; Descamps *et al*., 2008; Montes-Molina *et al*., 2008), affecting different biological parameters of insects and helping to regulate their populations (Descamps *et al.*, 2008, Acheuk *et al*., 2012). These biological compounds have a lower environmental impact than conventional insecticides since they break down rapidly in the environment and may be more specific (Chiasson *et al.*, 2004). However, most studies focus on analyzing the effect of these substances only on harmful phytophagous insects (Bruce *et al*., 2004; Malarvannan *et al*., 2009; Acheuk *et al*., 2012), ignoring the impact they may have on their natural enemies.

A wide variety of plants have currently been reported with insecticidal activity. The most promising of these may be *Azadirachta indica* (A. Juss.) (Meliaceae), commonly known as neem (Trujillo Ruiz *et al*., 2008; Brunherotto *et al*., 2010). Crude extracts with insecticidal properties have been obtained from plants of Verbenaceae, Asteraceae, Meliaceae, Rutaceae, among others (Pavela *et al*., 2008; Anita *et al*., 2012). The complexity of their composition gives them advantages for use in botanical preparations and they significantly delay the development of pesticidal resistance (Maja and Dorn, 2007). One species of Asteraceae is *Flourensia oolepis* Blake, commonly known as chilca, is endemic of Córdoba, Argentina. It is widely distributed in the Traslasierra Valley, and also in the hills of San Luis province (Sérsic *et al*., 2010). Among its secondary metabolites are flavonoids, compounds that can modulate the feeding and oviposition of insects visiting the host plant (Simmonds, 2001, 2003). Although the effects of certain active substances in plants of this genus are known (Diaz Napal *et al.*, 2010), the activity of its crude extracts has not yet been explored.

Considering this background, this study evaluated the effect of crude extract of *F. oolepis* on the oviposition behavior and reproductive parameters of female *Helicoverpa gelotopoeon* and the survival of the parasitoid adults *Archytas* sp. (Diptera: Tachinidae).

MATERIALS AND METHODS

Insects

Lepidoptera. A insectary was started from larvae of *H. gelotopoeon*, which were placed in plastic containers (8 cm diameter × 5.5 cm high) and fed with artificial diet based on chickpea flour (Fichetti, personal comm.), renewed every 48 h. To prevent mortality from cannibalism, individual 3rd instars were placed in similar boxes under controlled conditions of 25 ± 2ºC, 65 ± 5% RH, and photoperiod of 12:12 h (L:D). The resulting adults were placed in cardboard cylinders (24 cm high × 18 cm diameter), lined inside with sheets of absorbent paper, used as a substrate for oviposition (Schmidt *et al*., 1997). Adults were fed with a mixture of water, sugar, honey, methylparaben, ascorbic acid and vitamin B, provided in soaked cotton placed in a plastic container (4.5 cm high × 2 cm diameter) also lined with absorbent paper. The breeding cylinders and the food containers were checked and replaced daily to remove clutches (adapted from Bruce *et al.*, 2004). The eggs were placed in plastic boxes (11 cm wide × 5 cm high) until the emergence of the larvae.

Parasitoids. Tachinid pupae of the genus *Archytas* sp. were obtained from parasitized larvae and pupae of *H. gelotopoeon* collected in chickpea crops, and were placed in 30cm^2 cages until adult emergence. These were used for the bioassays.

Plant Extracts

These were obtained from the aerial parts of *F. oolepis* collected in the Traslasierra Valley, Córdoba, Argentina. To obtain the extract, 97 g of the dried and crushed plant were used and macerated in ethanol for 24 h (Diaz Napal *et al*., 2010). After evaporation of the solvent, the appropriate dilutions were made from the residue.

Oviposition Behavior

Choice tests were used to assess whether *F. oolepis* extract affects the oviposition behavior of the *H. gelotopoeon* adults. Half the surface of the breeding cylinders (base, walls and ceiling) and of the container with the food was sprayed using a graduated sprayer, with 0.1 mL of the chilca extract and the other half with solvent (acetone) (Dilawari *et al*., 1994). During spraying, to prevent contamination, the other half was covered by a plastic cloth. Two pairs of sexed pupae (2 ♀ and 2♂) were placed in each treated cylinder with treatments consisting of concentrations of 1, 2.5 and 5% and with 10 repetitions of each. Observations were made daily until the first oviposition was observed, and the eggs were counted thereafter under binocular microscope (Bruce *et al.*, 2004).

Survival and Reproductive Potential

To determine the effect of the extract on fecundity, fertility and longevity of adults of *H. gelotopoeon*, two pairs of sexed pupae per treatment were placed in each cylinder and five repetitions were made. Adults were fed with the diet described above, adding aliquots of extract at the same concentrations as used for the choice tests and using water/acetone as control. The variables measured were: day of emergence of each adult, day of first oviposition, number of eggs per cylinder, number of larvae hatched from each oviposition, and adult mortality (adapted from Schmidt *et al*., 1997). The period of pre-oviposition, oviposition and post-oviposition was determined, calculated as the average between days before, during and after oviposition (Bruce *et al.*, 2004). Fecundity was established as the number of eggs laid per female, and fertility was estimated as the number of larvae hatched. These data were recorded every 24 hours.

As adults of *Archytas* sp. in field feed on water and nectar (Tillman, 2008), intaking of the extract was used to assess their survival. For this propose, one tachinid was placed in each cage and fed with a 5% solution of honey soaked in a piece of cotton, placed in a plastic container. Aliquots of extract were added to this solution up to doses of 2.5% and 5%, and acetone (control) (adapted from Mitchell *et al.*, 2004). Each treatment was replicated four times, and survival was recorded daily.

Statistical analysis

A "t" test or Wilcoxon matched pairs test was used to analyze the choice test. The variables, *H. gelotopoeon* oviposition periods and parasitoid survival were analyzed with analysis of variance (ANOVA) or the non-parametric equivalent (Kruskal Wallis) (Infostat, 2010). Repeated measures ANOVA were used between treatments to compare the effect of the extract on lepidoptera fecundity and fertility.

RESULTS

Oviposition Behaviour

In the choice tests, only the 2.5% concentration gave significant differences in the number of eggs laid (t = 2.67, P = 0.025). The trend was maintained

Table 1. Effect of the different concentrations of *F. oolepis* extract on the oviposition (days) of *H. gelotopoeon*. Each value corresponds to the average of 5 responses (± SE)

Treatments	Pre-oviposition period	Oviposition period	Post-oviposition period
Water	2.5 ± 0.53^{b}	11.75 ± 0.53^{a}	2 ± 1.18^{a}
Acetone	3.4 ± 0.47^{ab}	12.4 ± 0.36^{a}	4.6 ± 1.06^{a}
1%	4.8 ± 0.47^{a}	10.4 ± 0.36^{ab}	3.2 ± 1.06^{a}
2.5%	3 ± 0.47^{ab}	7.4 ± 0.36^{b}	5.4 ± 1.06^{a}
5%	4 ± 0.47^{ab}	11.4 ± 0.36^{a}	2.8 ± 1.06^{a}

Different letters within each column indicate significant differences ($P \leq 0.05$, Tukeys test).

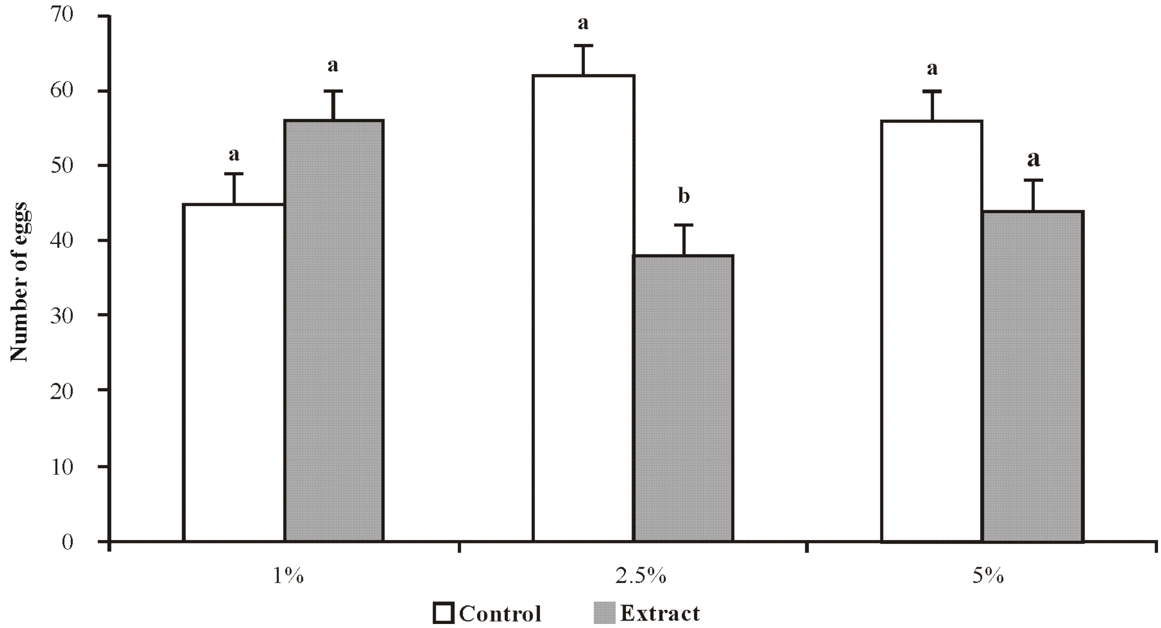

Fig. 1. Effects of F. oolepis extract on oviposition behavior of *H. gelotopoeon* females in choice test.

at the highest dose used, but the values were not significant (Fig. 1).

Survival of Adults and Reproductive Potential

The longevity of adults was not affected by eating treated food (females: $F = 1.77$; $df = 4, 19$; $P = 0.177$; males $F = 2.06$; $df = 4, 19$; P = 0.126). Increased survival of males was seen only with the 2.5% concentration ($t = -3.19$, $P = 0.012$) (Fig. 2).

In general, the different oviposition periods were not markedly affected by the intake of treated food, except for pre-oviposition which was up to twice as long with ingestion of 1% treated food compared to the water control ($F = 3.36$; $df = 4, 19$; $P = 0.031$). Also the oviposition period was significantly reduced compared to water control, when ingesting extract at 2.5% ($F = 7.29$; $df = 4, 19$; $P = 0.001$) (Table 1).

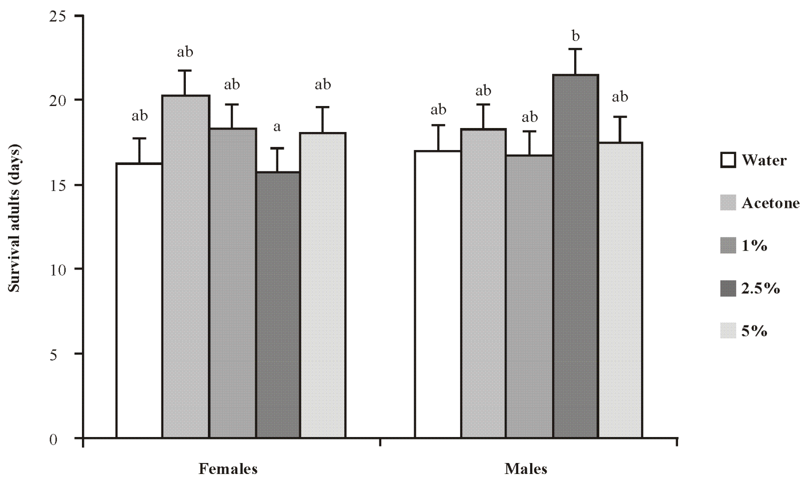

Fig. 2. Survival of *H. gelotopoeon* adults fed on different doses of *F. oolepis* extract and with water/acetone (controls).

The fecundity and fertility of *H. gelotopoeon* females were affected by intake of the extract. It significantly decreasing the number of eggs laid/ female during the course of the experiment ($F = 0.124$; $df = 6, 14$; $P < 0.001$). There was no interaction observed between time and dose ($F = 0{,}178$; $df = 24, 50$; $P = 0.191$) or among doses ($F = 0.828$; $df = 4, 19$; $P = 0.524$) (Fig. 3). The same trend was observed in larval emergence among doses, which decreased throughout the study ($F = 0.174$; $df = 6, 14$; $P < 0.001$) (Fig. 4).

When the tachinid parasitoids were fed with

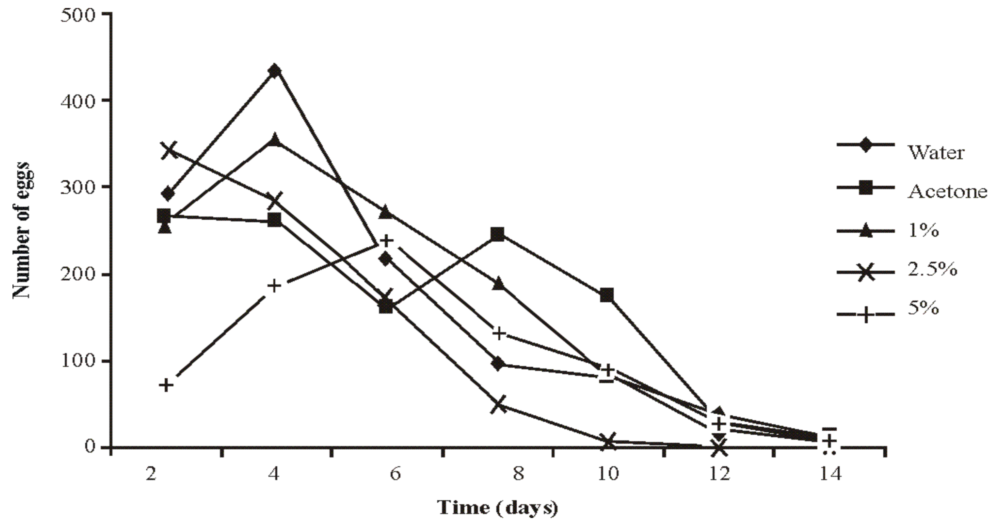

Fig. 3. Number of eggs laid/female pair of *H. gelotopoeon* fed with different concentrations of *F. oolepis* extract and water/acetone as control.

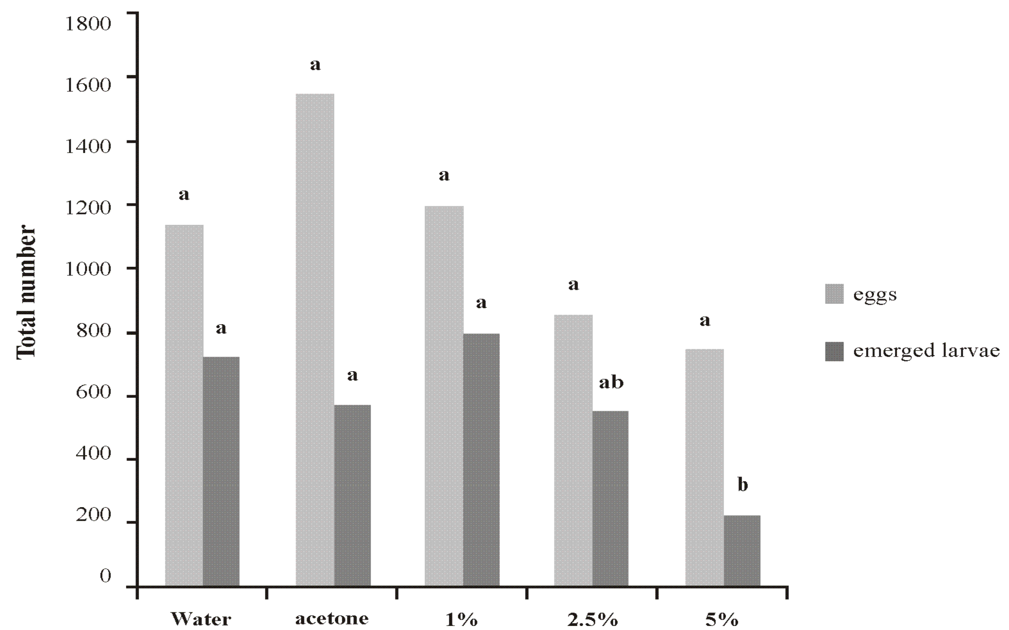

Fig. 4. Total number of eggs laid and hatched larvae of *H. gelotopoeon* females fed with different concentrations of *F. oolepis* extract and water/acetone as a control.

chilca extract, their survival was significantly lower at doses of 2.5% and 5% compared to the control (acetone) ($F = 106.58$; $df = 2, 7$; $P < 0.001$) (Fig. 5).

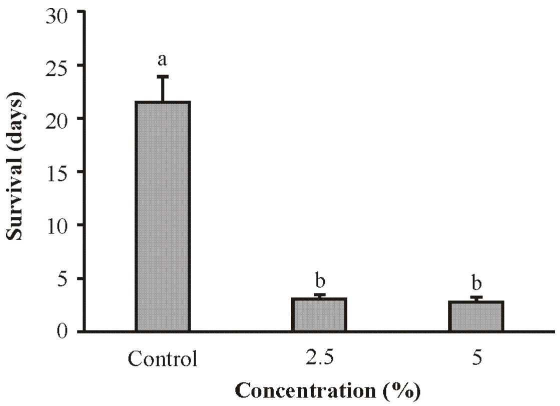

Fig. 5. Effect of *F. oolepis* extract on the survival of *Archytas* sp.

DISCUSSION

Fluorensia oolepis extract affected the oviposition behavior of *H. gelotopoeon* females, acting as a deterrent. This was seen with doses of 2.5% or higher, similar to that reported by Salunke *et al*. (2005) evaluating the action of partially purified flavonoids on the beetle *Callosobruchus chinensis* (L). The same effect was found by Gajmer *et al.* (2002), Bruce *et al*. (2004) and Malarvannan *et al*. (2009) using extracts of Meliacaeae on Noctuidae and Pyralidae. Martinez and Meneguim (2003) reported no differences between treatments using neem oil emulsion on *Leucoptera coffeella* Guérin-Meneville (Lepidoptera: Lyonetiidae).

In the no choice tests, male and female longevity was not affected by the different doses of chilca extract. While have been registered effects of crude extracts of other plant families on the longevity of Lepidoptera (Bruce *et al.*, 2004), we know nothing about the chemical similarities between these plant compounds. So at this point our discussion has been limited.

Overall, the *F. oolepis* extract did not alter the *H. gelotopoeon* oviposition periods, as Bruce *et al.* (2004) also found studying the effect of neem on lepidoptera. Exceptionally, pre-oviposition time increased when using the lowest dose and the oviposition period was shorter at a dose of 2.5%. We find no valid explanation for the effect of different doses on these periods. In this study, using water as control and extract-treated diet at 1% and 2.5% throughout the course of the experiment, it was observed that the highest oviposition occurred between the first and the fourth day. This was similar to that reported for other Noctuidae (Tisdale and Sappington, 2001).

Although fecundity and fertility were affected over the course of the experiment, only females who ingested food treated with the 5% dose laid half the number of eggs, and the number of hatched larvae was reduced by three times as compared to the water control. These responses match those observed by Salunke *et al*. (2005) using partially purified flavonoids at doses of 5%. Effects on fertility and fecundity were also reported by Schmidt *et al.* (1997), Bruce *et al*. (2004) and Nathan and Sehoon (2006) using Meliaceae extracts on lepidoptera. In addition, several studies have obtained similar results with different insects and plant extracts from different plants (Srivastava and Gupta, 2007; Shekari *et al*., 2008; Brunherotto *et al*., 2010; Acheuk *et al.*, 2012; Sahayaraj and Jeeva, 2012). Ingestion of crude extract of *F. oolepis* significantly decreased the survival of the parasitoid *Archytas* sp. Although this is not a usual situation in natural conditions, there is evidence that food intake treaty could affect the survival of parasitoids adults when that food is the unique resource for a prolonged time (Matter *et al*., 2002; Defagó *et al*., 2011). On the other hand, it is difficult to compare this with other results, because there are few publications on the effect of crude extracts of Asteraceae. However, many authors have reported adverse effects on the survival of predators and parasitoids of the Coleoptera and Hymenoptera when studying Meliaceae plant extracts (Viñuela *et al*. 2000; Iannacone and Lamas, 2003; Saber *et al*., 2004; Defagó *et al*., 2011). Moreover, studies with the endoparasitoid *Opius concolor* Szépligeti, fed with neem, showed a marked reduction in the adult longevity, in their ability to attack the host, and in progeny size (Viñuela *et al.* 2000). On the other hand, several authors have reported that neem does not interfere with the action exerted by natural enemies (Goudegnon *et al.*, 2000;

Viñuela *et al.* 2000; Akol *et al.*, 2003; Mitchell *et al.*, 2004; Tillman 2008). Nevertheless, Mikami and Ventura (2008) highlight the lack of compatibility studies between this compound and tachinid parasitoids. When designing strategies for pest management plans, it would be best to assess the conjunction of the different methods to use, as in some cases the use of natural extracts may be incompatible with biological control.

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