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Antifeedant and Toxic Effect of Crude Extract from *Flourensia oolepis* and their Impact on Nutritional Parameters of *Helicoverpa gelotopoeon*

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ABSTRACT: Botanical insecticides are one of the environmentally acceptable options for pest management. Extract of *Flourensia oolepis* (known as chilca), a plant endemic to the province of Córdoba, Argentina, has shown insecticidal activity. The aim of this work was to study the effect of crude extract of this *F. oolepis* on the nutritional parameters of *Helicoverpa gelotopoeon*, a polyphagous species recently reported as one of the most serious pests of chickpea. Choice tests were conducted using different doses of extract (1 to 10%) and acetone (control), and no-choice tests, feeding larvae for 10 days with chickpea leaves treated with extract (1 to 5%), with controls (water and acetone). We used three third instar larvae per replica and 8 repetitions of each. The variables measured were: consumption, fresh and dry weight of larvae, of feces and of the leaves given every 48 hours. We calculated the feeding inhibition and nutrition indices, and survival. In choice bioassays using the 10% dose, larvae preferred the control leaves, with the extract acting as a feeding inhibitor (92%). In the no-choice test, leaf consumption was markedly reduced with extract, affecting larval growth in a dose-dependent manner ($p < 0.05$), as well as feed utilization and lepidoptera survival.

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INTRODUCTION

The indiscriminate use of synthetic pesticides for pest control has caused serious social and environmental problems (Shekari et al., 2008). An environmentally acceptable alternative with proven effectiveness is the use of plant extracts, which have low persistence and are rapidly degraded (Gahukar, 2012). These crude extracts are usually mixtures of chemicals that produce a great variety of responses in different organisms (Begum et al., 2013). The complexity of their composition makes them suitable for use in ethnobotanical preparations and they have the advantage of considerably delaying the development of pest resistance (Alexenizer and Dorn, 2007).

Over the past 40 years, the search for such plants has been intensive and many leads have been identified from numerous plant species, the most promising belonging to the families Meliaceae, Asteraceae and Piperaceae (Shekari et al., 2008; Kathuria et al., 2013). Their secondary metabolites have different modes of action (pest repellents, growth retardants, antifeedants and lethal toxins), and having been studied against insects (Nathan, 2006; Gahukar, 2012). For the control of arthropods, particular attention has been paid to Asteraceae species, among these, chilca, *Flourensia oolepis* Blake (Diaz-Napal et al., 2009). Around 32 species of this genus of plants have been reported, growing from the southern U.S. to southern Argentina and Chile (Dillon, 1984).

Chickpea (*Cicer arietinum* L.) is important in the economy of Córdoba (Deromedis and Ochoa, 1974) and in recent years its production has expanded because of its export prospects (García Medina et al., 2007). One of the most important pests on this crop is the bollworm moth *Helicoverpa gelotopoeon* Dyar (Lepidoptera: Noctuidae), widespread in South America (Sharma et al., 2007). This polyphagous species act as defoliators, eating preferably buds and tender stems, but also flowers, pods and seeds (Igarzábal et al., 2011). Several *Helicoverpa* species have been documented as developing resistance to synthetic products (Murray et al., 2005), and so it is important to evaluate new substances for its management. In this paper, for the first time, we estimate the effect of the intake of *F. oolepis* crude extract on the feeding rate and growth parameters of *H. gelotopoeon.*

MATERIALS AND METHODS

Aerial parts of *F. oolepis* were collected from hills in the Traslasierra Valley of Córdoba, Argentina. These aerial parts were dried and crushed and macerated with ethanol for 24 h at room temperature. After removal of the solvent, the crude extract was obtained and used to make dilutions with acetone (Diaz Napal et al., 2009).

A hatchery for *H. gelotopoeon* was established from pupae, placed in cardboard containers under controlled conditions 27 ± 1ºC, 60 ± 5% RH and

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12:12 h L:D photoperiod. The moths emerging were fed a diet of sugar, honey, methyl paraben, ascorbic acid and vitamin B (adapted from Nathan et al., 2005). The eggs obtained were transferred to plastic containers. The larvae were fed a diet of chickpea flour, yeast, agar, ascorbic acid, sorbic acid and methyl paraben. For the assays, we used third instar larvae fed fresh chickpea leaves for 48 hours for adaptation before starting the bioassays.

Antifeedant activity was assayed using a choice test. Two leaves of *C. arietinum*, one treated and one untreated (acetone control), were placed in a Petri dish and a larvae placed equidistant from both and allowed to feed for 12 h. The dosages applied were 1%, 5%, and 10% dissolved in acetone. Twelve replicates were run for each treatment. The relative amounts of the treated and untreated substrate area eaten in each feeding choice test were estimated visually, always by the same operator. The feeding deterrence index (FDI) was calculated as (C-T)/(C+T) x 100, where C is the consumption of control leaf and T of the treated leaf (Díaz Napal et al., 2009).

In the no-choice test, three larvae were used in three treatments: extract (1%, 2.5% and 5%), and two in controls (water and acetone). Fresh leaves were sprayed with 0.1 ml of extract or acetone or water and dried at room temperature. After fasting for 4h, the fresh initial weight of the larvae was recorded, and they were placed in containers with the food. All weights were measured using a balance, accurate to 0.01mg. A total of 120 larvae (15 larvae/concentration, eight replicates) were allowed to feed on weighed quantities of extract-treated and -untreated *C. arietinum* leaves for 24h. The larvae weight difference gives the fresh weight gained during the study period. Three sample larvae were weighed, oven dried (48h at 60ºC), and re-weighed to establish the percentage dry weight. The leaves remaining at the end of each day were oven-dried and re-weighed to establish the percentage dry weight of the larvae diet. The quantity of food ingested was estimated by subtracting this leaf dry weight from the total dry weight of leaf provided. Feces were collected daily and weighed, and then oven-dried and re-weighed to estimate the dry weight of excreta. The experiment was continued for ten days and observations were recorded every 48 h.

Consumption, growth and post-ingestive utilization efficiencies (all based on dry weight) were calculated as follows: Consumption index (CI) = E/TA where E is the dry weight of food eaten, T is the duration of the experimental period and A is the mean dry weight of larvae during T; relative growth rate (RGR) = P/TA, where P is the dry weight gain of larvae; approximate digestibility (AD) = 100 (E-F)/E, where F is the dry weight of feces produced; efficiency of conversion of ingested food (ECI) = 100 P/E; efficiency of conversion of digested food (ECD) = 100 P/ (E-F) (Nathan, 2006). Survival, larval weight and leaf consumption over the experimental time were also calculated for each study dose.

Data from choice bioassays were analyzed with paired T test or its nonparametric equivalent (Wilcoxon). To determine the effect of the extract on the different nutritional indices and on survival, analysis of variance (ANOVA) or its nonparametric equivalent (Kruskal-Wallis) was performed. To compare dry weights of larvae, feces produced and food consumed per day repeated measures ANOVA between treatments was used.

RESULTS AND DISCUSSION

In choice tests *Flourensia oolepis* extract showed marked feeding deterrent activity on larvae of *H. gelotopoeon*, with a high feeding deterrence index at the highest concentration (10%) (Table 1). Doses of the extract below 10% had no marked effect on feeding behavior, which is similar to the data found by Nenaah (2013) using Coleoptera larvae and adults. On the other hand, García et al. (2007) observed a wide range of responses to *F. oolepis* essential oils, such as antifeedant activity on *Myzus persicae* (Sulzer) and *Leptinotarsa decemlineata* (Say), toxic action on *Tribolium castaneum* (H.) but null effect on *Rhopalosiphum padi* L.
Table 1

Various nutritional parameters were markedly affected in the no-choice tests. The intake of *H. gelotopoeon* larvae fed with leaves sprayed with different doses of extract throughout the experimental period varied significantly ($F_{5,26}$ = 523.208; p < 0.001). No interaction was recorded between time and concentration ($F_{20,87}$ = 1.333; p < 0.128) and, although larvae force-fed with 5% extract reduced intake, the differences between doses were not statistically significant ($F_{4,30}$ = 1.062; p = 0.392) (Fig. 1).Using extracts of *Melia azedarach* L. at the same doses on *Spodoptera eridania* Cramer moths, consumption dropped by 75% compared to control (Rossetti et al., 2008).
Fig. 1
Larvae fed with chickpea leaves showed marked weight loss throughout the course of the experiment ($F_{5,26}$ = 49.72; p < 0.001), with no interaction between time and dose ($F_{20,87}$ = 0.99; p < 0.475) or weight differences between the different treatments ($F_{4,30}$ = 0.782; p < 0.546) (Fig. 2). Rossetti et al. (2008) using paraíso extract found dose-dependent effects on weight and consumption of *S. eridania* larvae. However, Bruce et al. (2004) did not obtain differences in larval weight of Lepidoptera (Noctuidae and Pyralidae) between the different treatments using neem oil.
Fig. 2

There were significant differences in survival of control larvae and those fed with lower doses of extract compared to those which ate 5%, as from day four ($F_{4,35}$ = 5.62; p =0.001) (Fig. 3). Similar results were found by Defagó et al. (2011) and Rossetti et al. (2008) using Meliaceae compounds on *Colias lesbia* F. and *S. eridania* moths, respectively. This effect may be due to progressive poisoning over time produced by this and other compounds from Asteraceae, Meliaceae, etc. (Defagó et al., 2011; Shekari et al., 2008).

Fig. 3

The relative growth rate (RGR) was higher for the control than for the highest dose of extract ($F_{4, 30}$ = 3.24, $p \leq 0.02$) (Table 2), which reduced larval growth by 83%. There were no significant differences in the consumption index (CI) or approximate digestibility (AD) (Table 2). The same trend was observed with *M. azedarach* extract on Lepidoptera moths (Nathan, 2006; Rossetti et al., 2008). Similar responses have been found by researchers working with azadirachtin or different crude plant extracts (Wheeler and Isman, 2001; Sadek, 2003; Pavela et al., 2008; Ahmad et al., 2012). In this work there were no differences between treatments in the CI, matching the result obtained using another Asteraceae extract on *Xanthogaleruca luteola* (Müller) (Shekari et al., 2008). However, most of the studies have a variety of results, such as phagostimulant effects on larvae of *Spodoptera littoralis* (Boisduval) (Pavela et al., 2008) or antifeeding on other species of Lepidoptera (Nathan, 2006; Rossetti et al., 2008).

Table 2

The values of the conversion efficiencies of ingested (ECI) and digested (ECD) food decreased markedly with the 5% dose, compared to the control and the lower doses (ECI: $F_{4, 30}$ = 2.79; $p < 0.044$; ECD $F_{4, 30}$ = 2.93; $p < 0.037$) (Table 2). We observed a marked decrease in ECI (18.52%) and ECD (32.45%) between the control and the highest dose of the extract, as was also seen for *Agrotis ipsilon* (Huf.), *Spodoptera littoralis*, *S. frugiperda* (Smith), *S. litura* (Fabr.), *S. eridania*, and *Cnaphalocrocis medicinalis* (Guenée) (Lepidoptera) larvae fed with extracts from Meliaceae (Schmidt et al., 1997; Hernandez and Vendramin, 1998; Wheeler and Isman, 2001; Nathan, 2006; Rossetti et al., 2008). This could be because the energy destined for growth is redirected towards other paths, such as that responsible for detoxifying allelochemicals (Wheeler and Isman, 2001; Sadek, 2003). Although our results show a slight increase in AD between the highest dose of extract and the control, the differences were not significant. A similar response was recorded by Wheeler and Isman, (2001) using Meliaceae extract on larvae of *S. littura*. In contrast, other crude extracts generated a marked increase in this index for different species of lepidoptera moths (Koul et al., 2002; Nathan et al., 2005; Nathan, 2006). Reduced AD values were observed by Sadek, (2003), Pavela et al., (2008) and Shekari et al., (2008) and have been attributed to the insects using a greater proportion of the food to detoxify the extract and less for its own growth, causing a reduction in ECI and ECD (Shekari et al., 2008).

The effect of crude *F. oolepis* extract on larvae of *H. gelotopoeon* had not been assessed before and the biological activity observed is due to the mixture of chemical compounds present in it. The results obtained here suggest that the extract of this plant could be an option for the use of healthier compounds in programs of integrated pest management (IPM).

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Table 1 Antifeedant activities of *F. oolepis* extract against third instar larvae of *H. gelotopoeon.* Means of eight repetitions (±SE).

Concentrations (%)	*Control*	*Treatment*	*p*	*FDI (%)*
1	0.33±0.09	0.28±0.09	0.6352	8.96
5	0.43±0.1	0.28±0.1	0.5328	21.17
10	8.67±8.3	0.34±0.09	0.0028*	92.42

FDI (%) = Feeding deterrence index. * Within column differ significantly (Tukey's test, $p \leq 0.05$).

Table 2 Nutritional indices of third instar larvae of *H. gelotopoeon* after treatment with extract of *F. oolepis*. Means of eight repetitions (SE).

Treatment (%)	*RGR (mg/mg/day)*	*C I (mg/mg/day)*	*ECI (%)*	*ECD (%)*	*AD(%)*
Control	0.031(0.02)a	0.169 (0.04)a	18.52(0.01)a	32.45(0.01)a	57.08(7.48)a
Acetone	0.007 (0.01)ab	0.205 (0.03)a	3.72(0.01)ab	6.24(0.01)ab	59.57(6.82)a
1	0.020 (0.01)ab	0.186 (0.03)a	10.91(0.01)ab	19.98(0.01)ab	54.64(5.49)a
2.5	0.004 (0.01)ab	0.175 (0.03)a	2.72(0.02)ab	4.64(0.02)ab	58.71(13.76)a
5	-0.005 (0.02)b	0.182 (0.11)a	-2.90(0.02)b	-4.73(0.01)b	61.41(23.92)a

RGR: relative growth rate, CI: consumption index, ECI: efficiency of conversion of ingested food, ECD: efficiency of conversion of digested food, AD: approximate digestibility. Within columns, means followed by the same letter do not differ significantly (Tukey's test, $p \leq 0.05$).

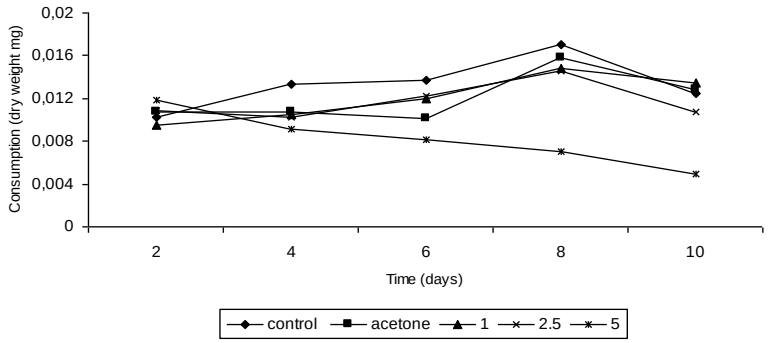

Fig1 Feed intake by larvae of *H. gelotopoeon* fed with leaves treated with different concentrations of *F. oolepis* extract (each point is the mean of 8 repetitions with 3 initial individuals each).

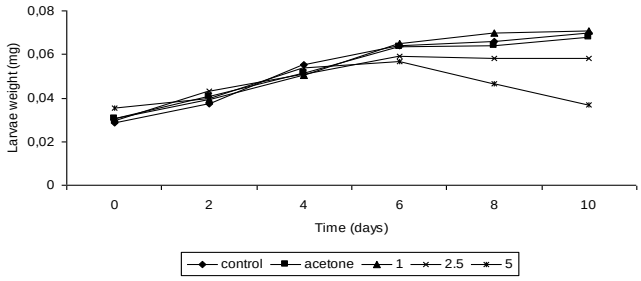

Fig 2 Mean fresh weight (mg) per larva of *H. gelotopoeon* fed with leaves treated with different concentrations of *F. oolepis* extract during the experiment period (each point is the mean of 8 repetitions with 3 initial individuals each).

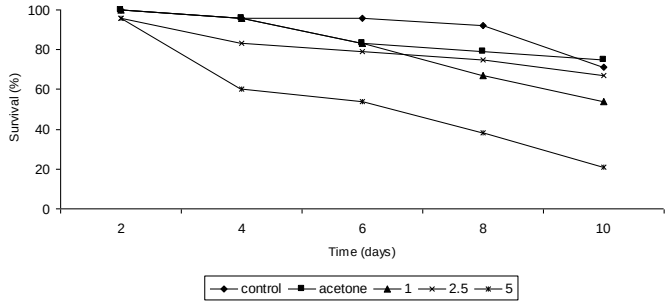

Fig 3 Survival of larvae of *H. gelotopoeon* fed with leaves treated with *F. oolepis* extract at different doses (each point is the mean of 8 repetitions with 3 initial individuals each).

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