Antifeedant effect of plant extracts on the poultry pest *Alphitobius diaperinus* (Coleoptera: Tenebrionidae): an exploratory study

FERNÁNDEZ, Nahuel F.[1], DEFAGÓ, María T.[1], PALACIOS, Sara M.[2] & ARENA, Julieta S.[1,*]

[1] Centro de Investigaciones Entomológicas de Córdoba (CIEC, UNC), Instituto Multidisciplinario de Biología Vegetal (IMBIV, CONICET-UNC) Universidad Nacional de Córdoba. Córdoba, Argentina.
*E-mail: julietaarena@conicet.gov.ar

[2] Laboratorio de Química Fina y Productos Naturales, IRNASUS-CONICET-Universidad Católica de Córdoba. Córdoba, Argentina.

Efecto antialimentario de extractos vegetales sobre la plaga avícola *Alphitobius diaperinus* (Coleoptera: Tenebrionidae): un estudio exploratorio

RESUMEN. En este estudio exploratorio se evaluó la actividad antialimentaria de nueve extractos etanólicos de plantas nativas de la región central de Argentina sobre *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), buscando alternativas de manejo de bajo impacto ambiental para esta plaga. Adultos de *A. diaperinus* fueron expuestos a alimento tratado con los extractos para evaluar si el comportamiento de alimentación y la supervivencia se ven alterados. La supervivencia de los adultos no fue afectada por ninguno de los extractos. Sin embargo, los extractos de *Gaillardia megapotamica, Vernonanthura nudiflora, Baccharis artemisioides, Lithraea molleoides*, y *Ambrosia artemisiifolia* generaron un efecto disuasivo fuerte (92-96%) sobre la alimentación del coleóptero. Los demás extractos evaluados afectaron ligeramente (50-55%) el consumo de alimento. Los extractos de *G. megapotamica* y *B. artemisioides* se administraron en el alimento del quinto estadio larval para determinar si afectan la supervivencia, el comportamiento de alimentación y el peso de las formas inmaduras. El extracto de *B. artemisioides* generó un marcado efecto disuasivo de la alimentación sobre las larvas (76%), durante la primera semana de experimentación, aunque el efecto se debilitó en el tiempo. Por su parte, *G. megapotamica* produjo a lo largo del ensayo un efecto disuasivo moderado (41-74%). Los extractos también afectaron el peso, la supervivencia y el número de exuvias larvales en relación al control.

PALABRAS CLAVE. *Baccharis artemisioides*. Bioplaguicidas. Comportamiento alimentario. Escarabajo de la cama. *Gaillardia megapotamica*.

ABSTRACT. In this exploratory study, the antifeedant activity of ethanolic extracts of nine plants native from central region of Argentina on *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) was evaluated, looking for low environmental impact management alternatives. Adults of *A. diaperinus* were exposed to food treated with the extracts to assess whether feeding behaviour and survival were altered. None of the extracts affected adult survival. However, the extracts of *Gaillardia megapotamica, Vernonanthura nudiflora, Baccharis artemisioides, Lithraea molleoides*, and *Ambrosia artemisiifolia* had a strong antifeedant effect (92-96%). The other extracts evaluated slightly affected (50-55%) the food consumption. The extracts of *G. megapotamica* and *B. artemisioides* were administered with the food to the fifth larval stage to assess whether they affect survival, feeding behaviour, and weight of immature forms. The *B. artemisioides* extract generated a marked deterrent effect of feeding on the larvae (76%) during the first week of experimentation, although the effect

weakened over time. On the other hand, *G. megapotamica* produced a moderate deterrent effect (41-74%) throughout the experiment. The extracts also affected weight, survival, and number of larval exuviae in relation to the control.

KEYWORDS. *Baccharis artemisioides*. Biopesticides. Darkling beetle. Feeding behaviour. *Gaillardia megapotamica*.

INTRODUCTION

Over the past two decades the poultry industry has experienced a rapid growth, with chicken meat and eggs providing high-quality protein, vitamins and oligoelements (Farrell, 2013). The warm and humid environment in which the birds are reared favours the development of different organisms including the darkling beetle *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) (Cecco et al., 2005). This cosmopolitan beetle causes serious economic losses, affecting birds, breeding facilities and farm workers. Larvae and adults act as mechanical vectors of bacteria, virus, and fungi, including *Bacillus* spp., *Campylobacter* spp., *Escherichia coli, Pseudomonas aeruginosa, Salmonella* spp., and *Staphylococcus* spp. (Roche et al., 2009; Agabou & Alloui, 2010; Ou et al., 2012; Crippen et al., 2018; Silva Soares et al., 2018), thus favouring the dispersion of pathogenic microorganisms. In addition, when they found in large numbers, injure the birds' skin causing a traumatic effect (Silva et al., 2005; Uemura et al., 2008). Furthermore, this insect damages breeding facilities (Japp et al., 2010), and causes allergies to people working on farms (Schroeckenstein et al., 1988).

The use of synthetic insecticides is the most common method to control *A. diaperinus*, with these products being applied by spraying the floor and walls before the replacement of the litter for the next breeding cycle to avoid direct contact with birds (Salin et al., 2003). However, loss of field efficacy of conventional insecticides and resistant *A. diaperinus* populations have been reported (Lambkin & Furlong, 2011; Arena et al., 2018; Hickmann et al., 2018), which has led to a great interest in the development of alternatives for managing this pest.

Plants produce several secondary metabolites which play an important role in defence against pathogens and herbivores. These compounds affect the behaviour of insects by acting as a deterrent or repellent, or alter development by affecting the digestion and assimilation of food, and some of them have a toxic effect (War et al, 2012). The use of botanical products as biopesticides have certain advantages over synthetic insecticides: they are biodegradable, have low persistence in the environment, and their toxicity to mammals and other vertebrates is relatively low (Gonçalves Marques et al., 2013). In addition, plant extracts are complex mixtures of compounds with different mechanisms of action, which reduces the probability of generating resistance (Akami et al., 2019).

Little is known about the effects of botanical extracts on *A. diaperinus*. Aqueous, acetonic, and ethanolic extracts obtained from *Ipomea fistulosa* Mart. ex Choisy (Convolvulaceae), *Datura fastuosa* L. (Solanaceae), *Eucalyptus citriodora* (Hook.) K.D. Hill & L.A.S. Johnson (Myrtaceae), *Helitropium indicum* L. (Boraginaceae), *Hedyotis corymbose* L. (Rubiaceae), and *Sapium indicum* Willd. (Euphorbiaceae) from Bangladesh have shown antifeedant effect on *A. diaperinus* (Kamruzzaman et al., 2005). In addition, when adult insects were sprayed with an aqueous extract obtained from *Azadirachta indica* A. (Meliaceae) and an ethanolic extract from *Ruta graveolens* L. (Rutaceae), mortalities of 98 and 61%, respectively, were produced (Marcomini et al., 2009).

Modifying the environment by providing inadequate sources of food could help reduce the harmful effects of *A. diaperinus* (Szczepanik et al., 2005). For example, the addition of natural compounds that affect the feeding behaviour of this insect could contribute to the decline of its populations on poultry farms. The aim of this work was to evaluate the effects of exposure of *A. diaperinus* to food treated with different extracts obtained from plants native from central region of Argentina on the feeding behaviour, development, and survival of this beetle.

MATERIAL AND METHODS

Insects

All the insects used in this study came from laboratory-reared colonies that were started with field-collected individuals from Colonia Caseros, Entre Ríos, Argentina. The coleopterans were maintained in plastic container under controlled temperature and relative humidity (25 ± 1 °C and 60 ± 5% RH) in darkness (Del Valle et al., 2016). A high-density polystyrene sheet was placed in the containers, which served as a site for the larvae to pupate. Insects were provided with a mixture of wheat bran and poultry feed (Rice & Lambkin, 2009).

Plant extracts

The extracts were provided by Fine Chemistry Laboratory of the Catholic University of Córdoba (UCC), which had been obtained from the aerial part of plants belong to families Asteraceae (*Gaillardia megapotamica* (Spreng.) Baker (UCCOR 127), *Trichocline reptans*

(Wedd.) Hieron (UCCOR 244), *Flourensia oolepis* Blake (UCCOR 135), *Vernonanthura nudiflora* (Less.) H. Rob (UCCOR 129), *Baccharis artemisioides* Hook. & Arn. (UCCOR 142), *B. coridifolia* DC (UCCOR 147), *Ambrosia artemisiifolia* L. (UCCOR 215), Polygonaceae (*Ruprechtia apetala* Wedd) (UCCOR 151), and Anacardiaceae (*Lithraea molleoides* (Vell.) Engl) (UCCOR 183). The specimens were collected in the province of Córdoba, Argentina, in the region of Chaco Serrano (30° 25' to 31° 59' S; 64° 21' to 65° 00' W), and deposited in the Herbarium of the School of Agricultural Sciences, "Marcelino Sayago", UCC. The vegetal material was dried at room temperature, crushed, and macerated during 48 hours with ethanol (95%); later it was filtered and the solvent evaporated under vacuum. The extracts were conserved at -5 °C (Diaz Napal et al., 2015). The extracts were diluted in ethanol to obtain the 10% concentration used in the assays, which generally has effects in insects (Marcomini et al., 2009).

Effects of plant extracts on *A. diaperinus* adults

Adults of *A. diaperinus* were fed with treated food in order to evaluate the effects of plant extracts consumption on feeding behaviour and survival. Wheat wafer discs (3 cm diameter) were used as test food (Kamruzzaman et al., 2005). The discs were treated with 150 µl of the extracts at 10% or ethanol (control) and dried for 24 hours at room temperature, after which the weight of each one was recorded (initial weight). Every disc and five unsexed adults 2-3 weeks old from the laboratory colony were placed in a Petri dish (6 cm diameter). All treatments were replicated six times, under controlled temperature (25 ± 1 °C) and relative humidity (60 ± 10%) in darkness. Mortality was checked daily and insects were considered dead when they showed no movement when touched with tweezers (Arena et al., 2020). After seven days the wafers were weighed again (final weight) to determine the amount of food consumed (FC). The antifeedant indexes (AI) were calculated as

$$AI = [(FC_C - FC_T) / (FC_C + FC_T)] \times 100$$

where FC_C is the amount of food consumed in control and FC_T is the amount of food consumed in the extract treatments (Diaz Napal et al., 2015). Extracts with AI ≥ 75% were considered strong antifeedants, while extracts with AI ≥ -75% were considered strong phagostimulants (Hassanali & Bentley, 1987).

Effects of plant extracts on *A. diaperinus* larvae

The extracts of *G. megapotamica* and *B. artemisioides* were used to evaluate the effects of ingestion on larvae of *A. diaperinus*. This assay was conducted following the methodology described above. All treatments were replicated 14 times using a 0.8 cm larva (corresponding to the fifth larval stage according to Hosen et al. (2004)) *per* dish in order to avoid cannibalism. Mortality and number of exuviae were registered daily for 28 days; in addition, larval weight

and wafer weight were recorded every seven days. The FC and AI were also calculated for each extract.

Statistical analyses

The variables amount of food consumed, larval weight and number of exuviae were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test, with a significance level used of $P < 0.05$, using the statistical software InfoStat (Di Rienzo et al., 2018). When the assumptions of normality and/or variance homogeneity were not achieved, Kruskal-Wallis tests were performed. The survival probabilities were estimated by the Kaplan-Meier method using the "survival" package (Therneau & Lumley, 2015) with the statistical software R version 3.5.1 (R Core Team, 2018).

RESULTS

Effects of plant extracts on *A. diaperinus* adults

Adults of *A. diaperinus* fed on ethanol-treated wafers consumed more food than those who were forced to feed on wafers treated with the extracts, with significant differences between treatments ($H = 42.81$; $P < 0.0001$) (Fig. 1). Beetles fed on wafers treated with extracts of *G. megapotamica* and *V. nudiflora* consumed 98 and 97% less, respectively, compared to the control. Insects forced to feed on food treated with *A. artemisiifolia*, *L. molleoides*, *B. artemisioides*, and *B. coridifolia* recorded intermediate consumes. Although feed consumption in the treatments with extracts of *R. apetala*, *T. reptans*, and *F. oolepis* was numerically lower than in the control, the differences were not significant.

The extracts of *G. megapotamica*, *V. nudiflora*, *A. artemisiifolia*, *L. molleoides*, and *B. artemisioides* had strong deterrent effects on *A. diaperinus* adults, while de *B. coridifolia*, *T. reptans*, and *F. oolepis* showed moderate deterrent effects. On the other hand, *R. apetala* extract caused a weak deterrent effect (Table I).

None of the evaluated extracts significantly affected the survival of the adults during the seven days of experimentation. The recorded mortality was 0.8% in the treatments with *B. coridifolia*, *G. megapotamica*, and *A. artemisiifolia*.

Effects of plant extracts on *A. diaperinus* larvae

According to the results observed in adults and the availability of extracts, we decided to evaluate the effects of two of them on the larvae. Thus, extracts of *G. megapotamica* and *B. artemisioides*, which had strong deterrent effects in adults, were used to assess the effects of long-term exposure of larvae to treated food. Larvae fed on wafers treated with *B. artemisioides* and *G. megapotamica* extracts consumed 83 and 62% less, respectively, compared to the control at seventh day of the experiment.

The consumption of larvae exposed to food treated with the extracts was statistically different from the control (day 7: $H = 19.26$; $P < 0.0001$; day 14: $H = 12.06$;
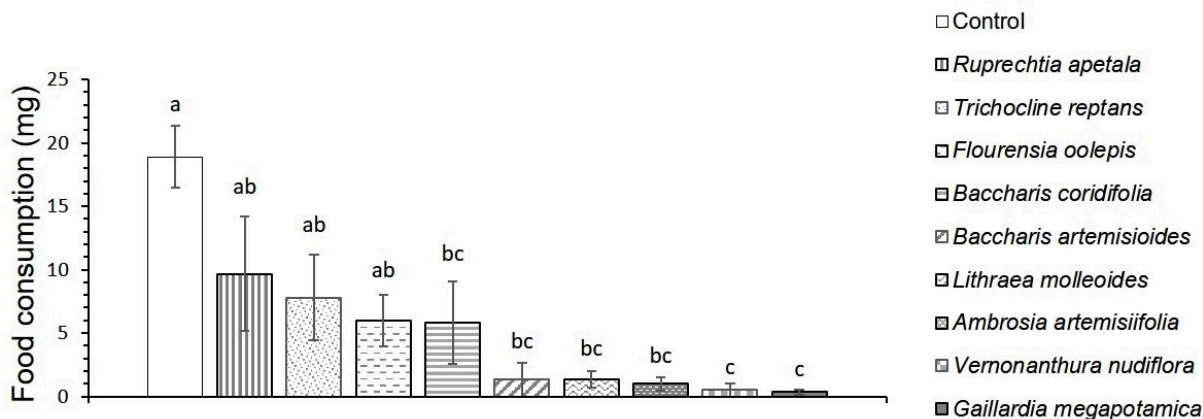
Fig. 1. Mean consumption (± SE) of wafers treated with different extracts or ethanol (control) by adults of *Alphitobius diaperinus* for 7 days (N = 6). Bars marked by different letters are significantly different according to LSD test ($P < 0.05$).
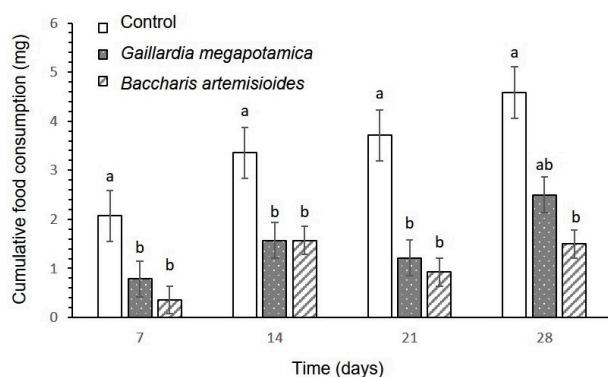

Fig. 2. Accumulated consumption (mean ± SE) by *Alphitobius diaperinus* larvae fed with wafers treated with extracts of *Gaillardia megapotamica* and *Baccharis artemisioides* or ethanol (control) (N = 14). Bars with the same letters at the same observation time do not differ significantly according to LSD test ($P > 0.05$).

Plant extracts	AI[1](%)	
Ambrosia artemisiifolia	94.4 ± 2.8	+++
Baccharis artemisioides	92.5 ± 7.5	+++
Baccharis coridifolia	72.2 ± 14.5	++
Flourensia oolepis	51.3 ± 11.3	++
Gaillardia megapotamica	96.4 ± 2.3	+++
Lithraea molleoides	92.7 ± 3.6	+++
Ruprechtia apetala	49.7 ± 16.5	+
Trichocline reptans	55.2 ± 12.9	++
Vernonanthura nudiflora	95.1 ± 4.9	+++

Table I. Antifeedant effect of plant extracts on *Alphitobius diaperinus* adults. [1]AI = Antifeedant Index; Mean values ± SE. AI ≥ 75% strong food deterrence (+++); 50% < AI < 75% moderate food deterrence (++); AI ≤ 50% weak food deterrence (+) (Hassanali & Bentley, 1987).

$P = 0.002$; day 21: $H = 17.44$; $P = 0.0001$). At day 28 only significant differences were observed between the consumption of larvae fed with *B. artemisioides* extract and the control ($F_{(2,23)} = 5.88$; $P = 0.009$) (Fig. 2).

After the first week of assay, the extract of *B. artemisioides* generated a strong deterrent effect, while that of the extract of *G. megapotamica* was moderate (Table II). During the time of assay, the deterrent effect of the *B. artemisioides* extract was attenuated, while that of *G. megapotamica* remained moderate after 28 days.

Although the larvae consumed the food offered, they experienced a decrease in weight throughout the experiment in both the treatments and the control. It is possible that the wheat wafers did not meet the nutritional requirements to ensure the growth of the larvae. However, differences in the larval weight were detected, being the larvae fed with wafers treated with

G. megapotamica extract the most affected in relation to the control and the treatment with extract of *B. artemisioides* (Fig. 3). The weight of the larvae that

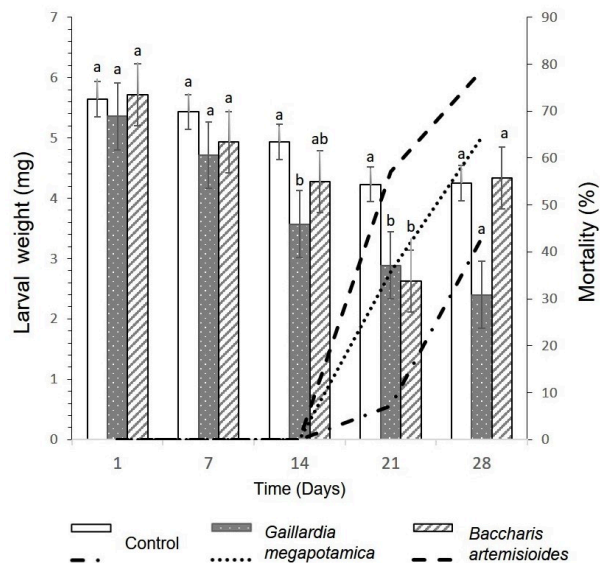**Fig. 3. Mean weight (± SE) (bars) and mortality (lines and/ or dots) of *Alphitobius diaperinus* larvae fed on wafers treated with extracts of *Gaillardia megapotamica* and *Baccharis artemisioides* or ethanol (control).** Different letters at the same observation time indicate significant differences in larval weight among treatments according to Tukey's test ($P < 0.05$).

received food treated with *G. megapotamica* extract was significantly lower than those that consumed wafers treated with ethanol and *B. artemisioides* extract on day 14 ($F_{(2,36)} = 6.72$; $P = 0.003$). At day 21 both extracts affected the larval weight showing significant differences with the control ($F_{(2,27)} = 5.87$; $P = 0.008$). Nevertheless, at day 28 the larvae that consumed *B. artemisioides* extract experienced an increase in weight, reaching values similar to the control. Larvae fed with wafers treated with *G. megapotamica* kept losing more weight in relation to the control and the treatment with *B. artemisioides* extract although no significant differences among treatments was recorded ($F_{(2,13)} = 1.73$; $P = 0.22$).

None of the extracts evaluated affected the larval survival during the first 14 days of experimentation (Fig. 3). Afterwards, the *B. artemisioides* extract was the most harmful, with 36% more dead larvae in relation to the control at the end of the assay. There were significant differences in the probability of survival being lower in the treatments with extracts in relation to the control (*Chisq*(2) = 9.3; $P = 0.009$) (Fig. 4). No significant differences were registered between the two extracts evaluated ($P = 0.7$).

The number of larval exuviae in the control was twice that of the extracts of *G. megapotamica* and *B. artemisioides* ($H = 7.99$; $P = 0.009$) (Fig. 5).

DISCUSSION

The effects of the exposure of *A. diaperinus* to food treated with extracts obtained from the nine selected plant species are reported for the first time. The results of this study revealed that the extracts affect the feeding behaviour of adults and larvae, as well as the weight, development, and survival of larvae. The reduction in food consumption and the low toxicity for adults suggest that these compounds may be unpalatable to this coleopteran (Glendinning, 2002; War et al., 2012). The effects of extracts from the plant species used in this study have been previously evaluated against other insects. For example, extracts of *F. oolepis, G. megapotamica*, and *L. molleoides* generated a total foraging inhibition of *Acromyrmex lundi* Mayr (Hymenoptera: Formicidae) in choice tests; whereas *R. apetala, B. artemisioides, V. nudiflora, A. artemisiifolia* produced intermediate foraging values, and *B. coridifolia* and *T. reptans* did not inhibit foraging activity (Diaz Napal et al., 2015). Plants from family Asteraceae have been particularly well studied due to the variety of secondary bioactive metabolites they possess, which makes them interesting sources of natural products to be used as bioinsecticides (Verdi et al., 2005; García et al., 2007; Padin et al., 2013). Contrary to what was observed in our study, compounds from *F. oolepis* had a strong antifeedant effect (98%) on *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae) (Diaz Napal et al., 2009), and, when applied by contact, caused 33% of mortality on adults of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) (García et al., 2007). The strong antifeedant activity of *V. nudiflora* recorded in this work against *A. diaperinus* adults could be attributed to the presence of sesquiterpene lactones, compounds with have shown marked deterrent effect on lepidopterans (Burnett Jr. et al., 1974).

Larvae of *A. diaperinus* consumed less food when it was treated with the plant extracts, mainly during the first week of experimentation. During the course of the assay, the antifeedant effect of *B. artemisioides* was weakened while that of *G. megapotamica* was maintained. In agreement with our results, larvae of *Epilachna paenulata* Germar (Coleoptera: Coccinellidae) avoided food treated with *B. artemisioides* extract in choice tests after 24 hours of experimentation, evidencing a strong deterrent effect (Del Corral et al., 2014). Similar results were obtained with larvae of this coccinellid when evaluating the extract of *F. oolepis* (Diaz Napal et al., 2009).

The extracts affected *A. diaperinus* larval weight, with a decrease observed throughout the experience in relation to the control. However, larvae exposed to wafers treated with *B. artemisioides* extract showed a weight gain after 21 days, reaching similar values to those of the control. According to these results, these individuals may have activated a metabolic pathway through which enzymes responsible for the

Plant extracts	AI[1](%)							
	Day 7		Day 14		Day 21		Day 28	
Baccharis artemisioides	76.3 ± 8.8	+++	52.4 ± 11.6	++	67.5 ± 11.5	++	31.2 ± 29.5	+
Gaillardia megapotamica	54.9 ± 9.7	++	41.2 ± 7.9	+	56.8 ± 8.3	++	74.3 ± 13.2	++

Table II. Antifeedant effect of *Baccharis artemisioides* and *Gaillardia megapotamica* extracts on *Alphitobius diaperinus* larvae. [1]AI = Antifeedant Index; Mean values ± SE. AI ≥ 75% strong food deterrence (+++); 50% < AI < 75% moderate food deterrence (++); AI ≤ 50% weak food deterrence (+) (Hassanali & Bentley, 1987).
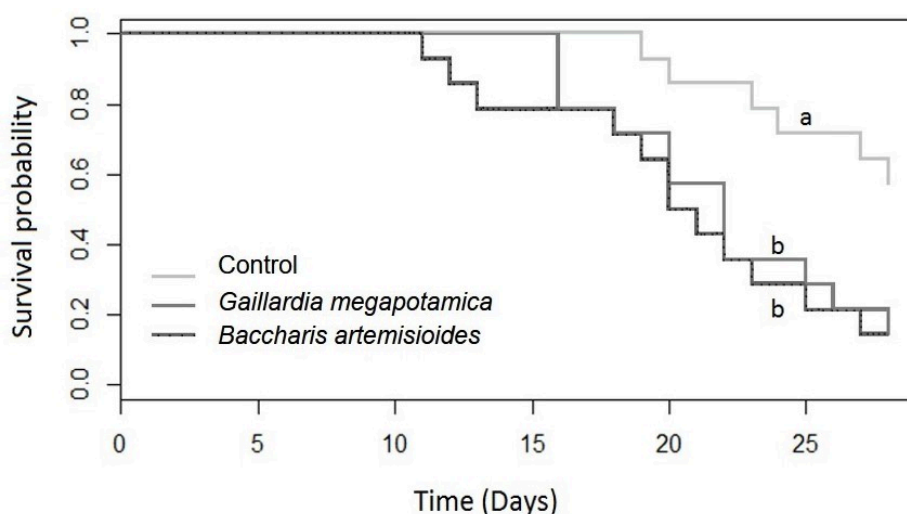

Fig. 4. Survival probability of larvae fed on wafers treated with extracts of *Gaillardia megapotamica* and *Baccharis artemisioides* or ethanol (control). Different letters indicate significant differences among treatments according to Log-Rank test ($P < 0.05$).

detoxification of secondary metabolites are expressed (Glendinning, 2002; War et al., 2012), or the active compounds may have degraded over time (Dayan & Duke, 2009). Larvae of *T. castaneum* fed with extracts obtained from other plant families behaved similarly to those recorded in this work (Jbilou et al., 2006).

The probability of survival of *A. diaperinus* larvae was lower in the treatments with extracts than in the control, which could be caused by starvation. Nevertheless, a toxic effect of the extracts cannot be dismissed since compounds from other plant families have adversely affected the larval survival of coleopterans, including *A. diaperinus* (Zorzetti et al., 2015; Ahmad et al., 2019).

The mean number of exuviae recorded was higher in the control than in the extracts of *G. megapotamica* and *B. artemisioides*, which indicates that these extracts would be affecting larval development, as observed by Jbilou et al. (2006).
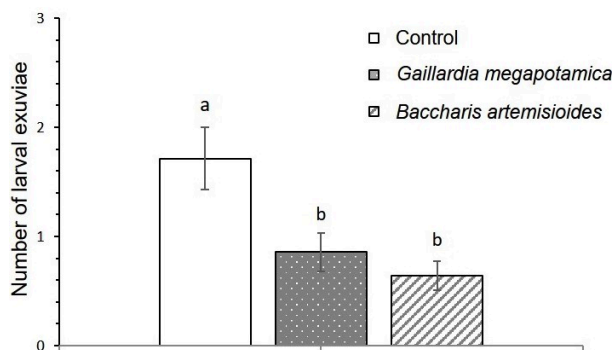

Fig. 5. Mean number of larval exuviae (± SE) obtained in tests with larvae fed on wafers treated with extracts of *Gaillardia megapotamica* and *Baccharis artemisioides* or ethanol (control). Different letters indicate significant differences among treatments according to Tukey's test ($P < 0.05$).

Secondary metabolites are biodegradable and of low toxicity to vertebrates, so they could be applied during the different stages of poultry farming, unlike synthetic insecticides. The extract of *G. megapotamica* maintained its deterrent activity throughout the experience, and that of *B. artemisioides* affected larval survival, both having promising characteristics as bioinsecticides.

In the future, evaluations of the extracts should be conducted using the birds' breeding litter and/or feed to assess survival and oviposition and feeding behaviours of *A. diaperinus* under conditions closer to those occurring on poultry farms. At the same time, consideration should be given to whether the food treated with these compounds affects the weight gain and oviposition capacity of the birds, as well as the organoleptic characteristics of the meat and eggs.

ACKNOWLEDGMENTS

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