EXTRACTS OF *Schinus molle* AND *Artemisia absinthium* AGAINST *Helicoverpa zea* ON FRESH EAR CORN IN ECUADOR

EXTRACTOS DE *Schinus molle* y *Artemisia absinthium* CONTRA *Helicoverpa zea* EN MAÍZ FRESCO EN ECUADOR

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RESUMEN

La alta presión de infestación de *Helicoverpa zea* (Boddie) en maíz (*Zea mays* L.) en Ecuador llevó a evaluar el efecto antialimentario y la mortalidad en laboratorio de larvas de tercer estadio expuestas a extractos en agua de hojas de *Schinus molle* (L.) y *Artemisia absinthium* (L.), al 100 y 50% de las concentraciones máximas obtenidas, puros y en mezcla con *Bacillus thuringiensis* (Berliner) var. *kurstaki* (Btk). Estos tratamientos se aplicaron también en un ensayo de campo. En el laboratorio, el extracto de *S. molle* tuvo efecto antialimentario medio (45 a 49%), mientras que el de *A. absinthium* no tuvo efecto. A 15 días después de la aplicación (DDA), los extractos puros o en mezcla con Btk causaron mortalidad baja (*S. molle*: 20 a 28%; *A. absinthium*: 24 a 40%; y en mezcla con Btk 33 y 28%, respectivamente). En esa evaluación, la mezcla de los extractos de *S. molle* y *A. absinthium* con Btk no aumentaron el efecto de la bacteria en las larvas. El extracto en agua de *S. molle* puro o en mezcla con Btk disminuyeron significativamente ($P \leq 0,05$) el número de larvas a la cosecha, pero el daño causado por las larvas supervivientes no difirió de aquel en el control no tratado. Los efectos insecticidas obtenidos aumentan las alternativas de control de esta importante plaga en maíz.

Palabras clave: Efecto antialimentario, efecto insecticida, extractos de plantas, gusano del choclo, *Helicoverpa zea*, *Zea mays*.

ABSTRACT

The high-pressure infestation of *Helicoverpa zea* (L.) in maize (*Zea mays* L.) in Ecuador led to evaluate the antifeedant effect and laboratory mortality of third instar larvae treated with water extracts from leaves of *Schinus molle* (L.) and *Artemisia absinthium* (L.) at 100 and 50% of the maximum concentrations obtained, which were applied alone and mixed with *Bacillus thuringiensis* (Berliner) var. *kurstaki* (Btk). A field trial was also conducted. In the laboratory, the *S. molle* extract had an intermediate (45 to 49%) antifeeding effect, while the *A. absinthium* extract had no effect. At 15 days after application (DAA), the extracts alone or in mixture with Btk caused low mortality (*S. molle*: 20 to 28%; *A. absinthium*: 24 to 40%; and in mixture with Btk 33 and 28%, respectively). On that evaluation, the combined application of *S. molle* and *A. absinthium* extracts with Btk did not increase the larvicidal effect of the bacteria. The water extract from *S. molle* applied alone or mixed with Btk

significantly decreased ($P \leq 0.05$) the number of larvae at harvest, but the damage caused by those surviving larvae did not differ from the untreated control. The insecticidal effects obtained represent new alternatives for the control of *Helicoverpa zea* in maize.

Key words: Antifeeding effect, *Helicoverpa zea*, insecticidal effect, plant extracts, corn earworm, *Zea mays*.

INTRODUCTION

Corn (*Zea mays* L.) (Poaceae) is grown everywhere in Ecuador, except in badlands and above 3000 m elevation with an average of 717,949 ton year⁻¹ of dry hard corn, mostly in the coastal provinces, and 43,284 ton dry soft corn, mainly in the Sierra provinces. From 2002 through 2009, ~100,000 ha of soft hard corn have been grown for local consumption mainly by farmers owning less than 2 ha (INEC, 2010).

Larvae of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) feed on many plants, and are common on fresh ears of corn in the USA, Mexico, and Central and South America (Artigas, 1994; Guevara and Granda, 2009). This species is active throughout the year in tropical and subtropical areas, but appears in the summer in temperate regions of the world. The life cycle can be completed in 30 d at a temperature of 34°C, and it can have 2-7 cycles in the United States (Capinera, 2001). Due to the climate of Ecuador, it does not have winter dormancy and it reproduces throughout the whole year (Guevara, 2014). Therefore, damage is critical in fresh corn because eggs are deposited on fresh styles of corn, and the hatching neonate larvae feed on them, affecting both pollination and grains (Capinera, 2001). Mature larvae leave the feeding site and drop to the ground and dig in the soil up to 8 cm to pupate (Araya and Cáceres, 2016). Damage from these larvae may be evaluated by their penetration in the ears of corn (Fig. 1).

In Ecuador, *H. zea* is controlled with contact insecticides, applied when the styles of corn appear, repeating their application after 7 d (Flores, 2010). Alternatives have been proposed to reduce the use of insecticides or to apply pesticides of low toxicity against pests and diseases (Peñaherrera, 2011).

Plant extracts have been used against crop pests for centuries and can help reduce the use of chemicals (Philogène et al., 2010). These natural products act and degrade rapidly, and also have selectivity and low impact on plants and beneficial insects. In addition, they do not favor development of resistant pests, and are rarely toxic for people and mammals (Regnault-Roger, 2004). In general, they affect the whole pest cycle, and may cause feeding repellency, mortality,

slow growth and development, block molting, and egg laying. In addition, they may be added to pesticides to strengthen their toxicity (Philogène, 2003).

Another alternative to reduce pesticides is the use of the bacteria *Bacillus thuringiensis* Berliner (*Bt*) for selective pest control. During spore formation, the *Bt* strains (e.g. Dipel™, Thuricide™) produce delta endotoxin, which kills caterpillars by paralysis (Sauka and Benintende, 2008). The basic pH of the digestive tract of caterpillars allows for spore germination and Cry toxin activation, which adhere to the intestinal epithelium; toxins induce cell lysis and death (Roh et al., 2007). The insecticidal effect of *Bt* var. *kurstaki* Berliner (*Btk*) against several noctuid pests (Kamel et al., 2010), and *H. zea* has been verified (Roh et al., 2007). Leaf extracts from boldo, *Peumus boldus* Molina (Monimiaceae), mixed with *Btk* have been studied against noctuid larvae, including *H. zea*, with variable results (Silva, 2010).

Foliar extracts of the pepper tree, or molle, *Schinus molle* L. (Anacardiaceae), a Peruvian native tree, have repellent and toxic effects on insects (Ferrero et al., 2006; 2007; Abdel-Sattar et al., 2009; Huerta et al., 2010). Absinthe wormwood, *Artemisia absinthium* L. (Asteraceae), has also toxic and antifeeding properties against mites and insects (Brudea, 2007; González-Coloma et al., 2012; Knaak et al., 2013). Both extracts are recommended in integrated pest management in central Asia (Maredia and Baributsa, 2007).

Given the importance of soft fresh corn and the increasing resistance to chemical pesticides against *H. zea* in Ecuador, the objective of this study was to evaluate the insecticidal and antifeeding effect of water extracts from leaves of *S. molle* and *A. absinthium*, singly and mixed with *Bt kurstaki*.

MATERIALS AND METHODS

Preparation of the extracts. The leaves of *S. molle* and *A. absinthium* were obtained in Riobamba City (1°40'00" S, 70°37'56" W), Ecuador, in June 2013. The extracts for the laboratory test and field trial were prepared in the General Laboratory of the School of Biology, College of Exact and Natural Sciences, Pontificia Universidad Católica

del Ecuador, Quito, Ecuador, following the methodology described by Chiffelle et al (2013). The laminae of both plants were separated from the raquis and allowed to dry under shade at $22 \pm 7^\circ\text{C}$ until they felt brittle (7 d and 15 d for *S. molle* and *A. absinthium*, respectively). Then they were ground with an electric mill, and sieved to obtain powder. Leaf powder samples of 100 g *S. molle* and *A. absinthium* were placed in 2000 mL glass beakers, and volumes of 500 and 1800 mL distilled water were added, respectively. The mixtures were set in a magnetic stirrer for 1 h at 37°C , and then for 23 h at room temperature, and allowed to rest until phase separation. The liquid phase was filtered through Whatman 1 filter paper. Then 12 mL were set on Eppendorf tubes and centrifuged at 1500 rpm during 15 min. The supernatant solutions were set in 1000 mL glass beakers. In total, volumes of 500 and 900 mL of pure extracts were obtained from 200 g of *S. molle* and *A. absinthium* leaf powders, respectively, and kept at 5°C . Solutions were 100% w/v.

Rearing of *H. zea*. About 300 larvae of various stadia were collected at a corn plot with no insecticide treatments during June, and taken to the laboratory in individual plastic boxes with corn styles. They were maintained at a $50 \pm 10\%$ RH, $22 \pm 7^\circ\text{C}$ and 12 h light photoperiod and fed daily with fresh grains until they pupated. The pupae were set on vials with soil after solarization, and humidified every 3 d until adult emergence.

The same number of males and females (the pupae were separated previously by morphology; see Waldbauer et al., 1984) were set to mate in glass containers with a ventilated lid. A corn ear

with exposed fresh styles and 10% w/v honey was offered to feed the adults in a small cotton swab, and replaced daily. The eggs laid were set in glass vials in the chamber described above and hatching occurred at ~ 5 d. The emerging larvae were immediately fed with fresh alfalfa sprouts until they grew to the 2nd stadium, when they were fed with fresh corn grains until they were used in the tests. When they reached the 3rd larval stage, ~ 15 cm long and 7 d after hatching (Zúñiga et al., 2011), they were fasted 6 h before submitting them to the corresponding treatments to homogenize feeding.

Test of the antifeeding effect bioassay. The treatments used (Table 1) were evaluated in the antifeeding effect test of *S. molle* (100 and 50% w/v) and *A. absinthium* (100 and 50% w/v) using 10 mL of the solutions.

The extracts were applied alone and combined with *Btk* 50% to verify any synergistic effect with the bacterium, as reported by Arretz et al. (1976), Isman et al. (2006) and others (see below).

The study by Rossetti et al. (2008) was used as a model, with some modifications. A completely randomized design with 4 treatments was used in a free choice test with 15 replicates (1 Petri dish each). The experimental unit was one 3rd stadium larva in a Petri dish with 1 fresh corn grain, which was previously immersed in the treatment, and 1 untreated fresh corn grain (control). The fresh weight consumed of the treated and control grains were measured at 24 h using a precision scale (Boeco BBL-52, Hamburg, Germany).

For application, 15 fresh corn grains were immersed for 1 min in 10 mL of each extract and

Table 1. Treatments evaluated in the laboratory bioassay for their insecticidal effect on 3rd stadium *H. zea* larvae, active ingredients and volume/concentrations.

Treatments	Active ingredients	Volume used (mL) in the bioassay	
		on 3 rd stadium larvae	Concentrations
<i>S. molle</i> 100% v/v	Plant extract	10	---
<i>S. molle</i> 50% v/v	Plant extract	10	---
<i>A. absinthium</i> 100% v/v	Plant extract	10	---
<i>A. absinthium</i> 50% v/v	Plant extract	10	---
Dipel TM WG (<i>Btk</i>) 100%	<i>B. thuringiensis</i> var. <i>kurstaki</i>	---	1.8 g L ⁻¹
Dipel TM WG (<i>Btk</i>) 50%	<i>B. thuringiensis</i> var. <i>kurstaki</i>	---	0.9 g L ⁻¹
<i>S. molle</i> 100% v/v + <i>Btk</i> 50%	Plant extract + <i>Btk</i>	10	0.9 g L ⁻¹
<i>A. absinthium</i> 100% v/v + <i>Btk</i> 50%	Plant extract + <i>Btk</i>	10	0.9 g L ⁻¹
Nockeo TM	Thiametoxam and lambdacyalothrin	---	0.6 mL L ⁻¹
Control	None	---	---

concentration in 50 mL glass vials. Thereafter, these grains were allowed to dry at room temperature on filter paper for ~5 min. The grains used in the treatments and the control were weighed, and the Petri dishes were marked correspondingly. Due to the fact that larvae bite or eat one another, only one larva was placed in the center of the Petri dish for each treatment. To correct for weight loss by dehydration over 24 h, another 15 grains were set aside in a Petri dish.

Corn consumption (%) was evaluated once a day after application (1 DAA) by recording the weight of the treated and untreated grains - corrected for weight loss by dehydration - to establish the weight of the grain consumed by the 3rd stadium *H. zea* larvae. The Antifeeding Inhibition Index (AII; Defagó et al., 2006) was determined with the formula $AII\% = (1 - T/C) \times 100$, where T and C are the mean consumptions of the treated and control grains, respectively. The weights obtained were analyzed with non-parametric statistics, using Wilcoxon test for ranked paired samples with InfoStat statistic software version 2102 (Di Rienzo et al., 2012).

Laboratory test of the insecticide effect. The scale used to assess the level of damage in the test of the insecticide effect is presented in Fig. 1.

To improve the application and wetting of the grains, the pH of all extract solutions was reduced to ~5 with the commercial product Indicate 5TM as recommended by the manufacturer (Ecuquímica, Ecuador) to enhance insecticidal results.

DipelTM WG (Valent BioSciences Corporation, USA, distributed by ANASAC, Chile) is a microbial biological insecticide containing the bacteria *B. thuringiensis* var. *kurstaki* (*Btk*) as active ingredient, in a formulation of water soluble granules that acts upon ingestion. NockeoTM is an insecticide used in agriculture (Parijat Industries, India, imported and distributed by El Agro, Ecuador), whose active ingredients are thiamethoxam (141 g L⁻¹), a systemic neonicotinoid with broad spectrum action, and lambda-cyhalothrin (106 g L⁻¹), a contact pyrethroid. Indicate 5TM (Marketing ARM International, imported and distributed by Ecuquímica, Ecuador) is a liquid wetting co-adyuvant that regulates the pH of the water used for spraying; it also acts as a surfactant that reduces surface tension of a solution.

A completely randomized design was used with 10 treatments (including the untreated control and NockeoTM) and 5 replications (10 Petri dishes were a replicate, each with only one 3rd stadium larva to avoid predation and damage to other larvae). The experiment used ten 3rd

stadium larvae with 10 fresh corn grains on which the corresponding treatments were applied by immersion for 1 min in 10 mL of the respective solution. Subsequently, they were set to dry at room temperature for 7 min. After a 6 h fast, a 5-10 mm long larva was placed in a Petri dish with the treated grains; the mortality rate was recorded for each treatment at 24 h and daily for 15 d. Food was replaced daily with untreated fresh corn grains during the 15 d evaluation period. The percentages of cumulative larval mortality were normalized with Bliss transformation. After an ANOVA, significant differences were identified with Tukey's test for mean comparisons. Statistical analyses were performed using the InfoStat version 2012 (Di Rienzo et al., 2012).

Field trial. A commercial corn crop was grown in Chambo County, Chimborazo Province, Ecuador (1°42'32" S; 78°35'52" W), at 2,500 m elevation with an average temperature of 15°C and surrounded by a tree tomato plantation (*Solanum betaceum* Cav.) and a cabbage (*Brassica oleracea* L.) crop. The corn crop used to evaluate the effect of the plant extracts combined with *Btk* was established on a 2,457 m² field in the Centro Agrícola del Cantón Chambo, from June to November 2013, following recommendations of Peñaherrera (2011), with some modifications.

The corn var. INIAP-101 Blanco Harinoso Precoz, recommended for the area, was sown on June 14, on a site furrowed at 80 cm between rows. Fertilizers, plant growth promoters, herbicide, and atrazine herbicide were applied as customary by local corn growers at early crop stages (products, application dates and rates appear in Guevara (2014), and should not interfere with treatments on fresh styles. A randomized complete block design with 10 treatments and 5 replications was used. Each plot or replication was a 2 m wide x 2.5 m long experimental unit, with 4 rows and ~56 plants, approximately 112 style groups and 20 ears of corn harvested to record the number of larvae found and to evaluate damage after treatment application. The plots were separated by one row. The treatments (the same as in the laboratory; Table 1) were applied starting with the appearance of the first ear styles (24 September) on the plants through November 8, every 4 d (13 sprays in total), using 200 mL hand sprayers to wet each style group with a 0.4 mL solution of the treatment prepared for application.

On November 12, twenty corn ears were harvested at random on each experimental plot and taken to the laboratory in marked bags to evaluate the number of larvae and damage level. To evaluate the presence of *H. zea* larvae in the treated plots, the healthy larvae found on 20

ears of corn were counted in each replication of each treatment. The means were analyzed with an ANOVA, and when significant differences appeared, the Tukey test was applied at 95% confidence level. Data were analyzed using InfoStat software version 2012 (Di Rienzo et al., 2012).

Simultaneously, 20 ears of corn were checked to determine larval damage in the experimental plots (Fig. 1). Levels 2 through 5 were included in the evaluation of the percentage of damaged ears. Level 1 was not included because it affected the styles but not the grains, and thus market value was not affected. However, the percentage of ears of corn damaged and penetrated included levels

1 through 5. The intensity of damage (IDD) was calculated with the formula in Townsend and Heuberger (1943):

$$IDD_{N(1-5)} = (N1 \times 1 + N2 \times 2 + N3 \times 3 + N4 \times 4 + N5 \times 5) / n$$

where $N_{(1,...,5)}$ is the number of ears of corn found at damage levels (1,...,5), and "n" is the number of ears of corn evaluated (20). The means expressed in percentage were normalized with Bliss transformation. ANOVA was used for data analyses. When significant differences appeared, Tukey test was applied at 95% confidence level. Mean differences were determined using InfoStat software version 2012 (Di Rienzo et al., 2012).

Fig. 1. Damage levels evaluated for *H. zea* on fresh ear of corns at harvest. 0: Undamaged; 1: Slightly damaged styles; larvae dig down to 1 cm from the apex, but do not reach or affect grains; 2: Moderate ear damage, 2 cm down from the apex; small gallery, 1 to 3 grains affected; 3: Intermediate damage; penetration down to 3 cm from the apex, large gallery, 4-6 grains affected; 4: Severe damage, penetration down to 4 cm, 7-9 grains affected; 5: Very severe damage, penetration > 5 cm, 10 or more grains affected. Levels were modified from Arretz et al. (1976).

RESULTS AND DISCUSSION

Antifeeding effect. The antifeeding results of 3rd stadium *H. zea* larvae after exposure to the treatments applied by immersion of soft fresh corn grains in the laboratory bioassay are presented in Table 2.

The water extracts from the leaves of *S. molle* and *A. absinthium* caused different antifeeding effects. The *S. molle* treatments had a similar antifeeding effect, between 45 and 49%, while no effect occurred with the *A. absinthium* extract at both concentrations.

However, the antifeeding effect obtained with the *S. molle* extract was not sufficient to produce significant differences in larval consumption of the treated and untreated grains according to Wilcoxon's test. These results confirm the diverse antifeeding effects of several compounds reported in the literature (i.e., Arretz et al., 1976; Defagó et al., 2006; Isman, 2006; Abdel-Sattar et al., 2009; González-Coloma et al., 2012).

Insecticidal effect in the laboratory. The mortality rate of 3rd stadium larvae exposed to the treatments applied by 1-min immersion on fresh corn grains in the corresponding solutions are presented at 1, 8, and 15 DAA in Table 3.

At 1 DAA, thiamethoxam + lambdacyhalotrin produced 76% mortality, with significant differences with respect to the untreated control, followed by the *A. absinthium* 50% treatment with 32%. At 8 DAA, cumulative mortality due to thiamethoxam + lambdacyhalotrin increased to 92%, showing significant differences with the control. The *Btk* 100% and *A. absinthium* 50% w/v treatments followed in efficacy, with 52 and 40% mortality, respectively. At 15 DAA, thiamethoxam + lambdacyhalotrin and *Btk* 100% caused the greatest mortality compared to the control treatment, reaching rates of 92 and 64%, respectively. In addition, the treatments *Btk* 50%, *S. molle* 100% + *Btk* 50%, and *A. absinthium* 50% w/v were statistically different from the control, with mortality rates of 48, 44, and 40%,

Table 2. Effect of the water extracts from leaves of *S. molle* or *A. absinthium* at different concentrations on 3rd stadium *H. zea* larval feeding in a free choice selection test in the laboratory.

Extracts	Corn grain consumed (% ± SE)		Antifeeding effect (%)
	Untreated control	Treated grains	
<i>S. molle</i> 100%	4.89 ± 0.01 a	2.57 ± 0.00 a	45.16
<i>S. molle</i> 50%	6.89 ± 0.01 a	3.26 ± 0.01 a	48.78
<i>A. absinthium</i> 100%	7.56 ± 0.01 a	8.53 ± 0.01 a	0.00
<i>A. absinthium</i> 50%	3.84 ± 0.00 a	8.07 ± 0.00 a	0.00

Means in a column with the same letter are not significantly different ($P \leq 0.05$), according to a Wilcoxon test for paired samples.

Table 3. Mortality (% ± SE) of *H. zea* larvae by effect of the water extracts from *S. molle* or *A. absinthium*, applied alone and mixed with *Btk* at 1, 8, and 15 DAA in the laboratory.

Treatments	Days after application (DAA)		
	1	8	15
<i>S. molle</i> 100%	4.0 ± 2.8 c	16.0 ± 5.3 bc	20.0 ± 4.5 bc
<i>S. molle</i> 50%	0.0 ± 0.0 c	24.0 ± 10.4 bc	28.0 ± 9.6 bc
<i>A. absinthium</i> 100%	12.0 ± 3.5 bc	24.0 ± 6.3 bc	24.0 ± 10.4 bc
<i>A. absinthium</i> 50%	32.0 ± 7.2 b	40.0 ± 7.2 b	40.0 ± 6.3 b
<i>Btk</i> 100%	8.0 ± 3.5 c	52.0 ± 3.5 ab	64.0 ± 2.8 ab
<i>Btk</i> 50%	4.0 ± 7.2 c	32.0 ± 3.5 bc	48.0 ± 5.7 b
<i>S. molle</i> 100% + <i>Btk</i> 50%	0.0 ± 0.0 c	32.0 ± 3.5 bc	44.0 ± 5.3 b
<i>A. absinthium</i> 100% + <i>Btk</i> 50%	4.0 ± 2.8 c	20.0 ± 6.3 bc	28.0 ± 3.5 bc
Thiamethoxam + lambdacyhalotrin	76.0 ± 6.3 a	92.0 ± 3.5 a	92.0 ± 3.5 a
Untreated control	0.0 ± 0.0 c	0.0 ± 0.0 c	4.0 ± 2.8 c

Means in a column with the same letter are not significantly different ($P \leq 0.05$), according to Tukey tests.

respectively (Table 3). These results with the insecticide formulation and *Btk* are comparable to those in the literature (e.g. Burkness et al., 2008, and Aguilar-Medel et al., 2007, respectively), but do not reveal a synergistic effect when mixed with *Bt*, as reported by Arretz et al. (1976), and Narkhede et al. (2017).

Effect of the field treatments on the number of larvae found on the ears of corn and damage level. The number of larvae of *H. zea* at different development stages found during the evaluation of fresh ears of corn in the field is presented in Table 4.

The treatments with thiamethoxam + lambdacyhalotrin, *S. molle* 100% v/v + *Btk* 50%, *Btk* 100%, and *S. molle* 100% w/v presented significant differences with respect to the control in the means of larvae observed at harvest. In terms of damaged ears of corn, damaged and penetrated ears, and damage intensity, only the thiamethoxam + lambdacyhalotrin treatment resulted in significantly less damage than the untreated control. The treatments with extracts applied alone or combined with *Btk* were not different from the control (Table 4).

Antifeeding effect in the field trial. The antifeeding effect of the *S. molle* leaf extracts at 100 and 50% were 45.16 and 48.78%, respectively. These values were relatively low compared to the antifeeding effect of water extracts of *S. molle* leaves on adults of the elm leaf beetle *Xanthogaleruca luteola* (Muller) (Coleoptera: Chrysomelidae) as different concentrations (2.5, 3.0, 4.3, and 5.6%

w/v) caused an antifeeding effect up to 98.32% (Huerta et al., 2010). Major oil components of *S. molle* dried leaves by hydrodistillation were 27.1% cubenol, 15.3% caryophyllene oxide, and 12.4% spathulenol (Dos Santos et al., 2015).

The antifeeding effect of the *S. molle* extract at both concentrations on 3rd stadium *H. zea* larvae was quantitatively similar to that of Silva et al. (2013) on the antifeeding effect of *P. boldus* leaf extracts on 3rd stadium *H. zea* larvae. With *P. boldus* leaf extracts at 0.50 to 8% w/p, the antifeeding effect varied from 37.7 to 57.3%, with no significant differences between the concentrations.

Insecticide effect in the field trial. The water extracts of *S. molle* and *A. absinthium* caused a gradual increase of the cumulative mortality during 15 d. The effectiveness of these extracts should be complemented with studies of their effect on the entire lifecycle of *H. zea*. According to Silva et al. (2013), when the larvae were treated with the water extract of *P. boldus*, the size, diameter, weight, and the number of larvae that reached the pupal stage decreased with the increase in concentration of the extract. Besides, these larvae took longer to start pupating.

The water extract from *S. molle* at 100% caused 16% mean mortality at the 8 d evaluation. This value is lower than that produced with *S. molle* water extracts at 5.6% w/v onto *X. luteola* adults, 27.8% at the 4 d evaluation (Huerta et al., 2010), and much smaller than the 67 and 63% mortality obtained with water extracts from new and mature leaves, respectively, onto 3rd stadium larvae of

Table 4. Means ($\pm$ SE) of the number of *H. zea* larvae on 20 ears of corn sprayed every 4 d from style appearance on "Suave INIAP 101" soft fresh corn, damaged ears, damaged and penetrated ears, and damage intensity at harvest, in Chambo County, Chimborazo Province, Ecuador.

Treatments	Larvae on 20 ears of corn	Damaged ears of corn (%)	Damaged and penetrated ears of corn (%)	Damage intensity
<i>S. molle</i> 100%	4.8 $\pm$ 1.3 bc	50.0 $\pm$ 6.9 a	64.0 $\pm$ 6.8 a	2.0 $\pm$ 0.3 a
<i>S. molle</i> 50%	5.4 $\pm$ 0.8 abc	57.0 $\pm$ 6.4 a	69.0 $\pm$ 5.3 a	2.2 $\pm$ 0.3 a
<i>A. absinthium</i> 100%	5.0 $\pm$ 1.2 abc	56.0 $\pm$ 6.9 a	71.0 $\pm$ 4.9 a	2.1 $\pm$ 0.2 a
<i>A. absinthium</i> 50%	5.6 $\pm$ 1.0 abc	52.0 $\pm$ 4.9 a	61.0 $\pm$ 5.6 a	2.0 $\pm$ 0.2 a
<i>Btk</i> 100%	4.4 $\pm$ 0.5 bc	58.0 $\pm$ 6.8 a	65.0 $\pm$ 5.0 a	2.0 $\pm$ 0.3 a
<i>Btk</i> 50%	5.6 $\pm$ 0.9 abc	68.0 $\pm$ 8.3 a	73.0 $\pm$ 7.5 a	2.8 $\pm$ 0.5 a
<i>S. molle</i> 100% + <i>Btk</i> 50%	3.4 $\pm$ 0.9 c	46.0 $\pm$ 4.9 a	55.0 $\pm$ 4.7 a	1.8 $\pm$ 0.2 a
<i>A. absinthium</i> 100% + <i>Btk</i> 50%	8.6 $\pm$ 1.0 ab	54.0 $\pm$ 7.3 a	68.0 $\pm$ 6.8 a	2.4 $\pm$ 0.4 a
Thiamethoxam + lambdacyhalotrin	2.6 $\pm$ 0.9 c	14.0 $\pm$ 4.0 b	21.0 $\pm$ 4.3 b	0.6 $\pm$ 0.1 b
Untreated control	10.0 $\pm$ 1.8 a	41.0 $\pm$ 7.0 a	61.0 $\pm$ 6.0 a	1.8 $\pm$ 0.3 a

Means in a column with the same letter are not significantly different ($P \leq 0.05$), according to Tukey tests.

X. luteola with the 4.3% w/v concentration by Chiffelle et al. (2013).

The results obtained indicate a slight effect of the water extracts from *S. molle* and *A. absinthium* on larval mortality of *H. zea*. Only 20 and 28% cumulative mortality occurred with *S. molle* 100 and 50%, respectively, while rates of 24 and 40% were recorded at 15 DAA with *A. absinthium* 100 and 50% mortality, respectively. A low mortality rate was also reported by Silva et al. (2013), who obtained 30% mortality with the highest concentration (8% w/v) of water extract from *P. boldus* leaves against *H. zea* in laboratory tests.

The interaction of the *S. molle* or *A. absinthium* extracts with *Btk* did not increase the effect of the bacterium alone. In addition, Silva (2010) did not find synergism between *P. boldus* extract and *Btk* on the mortality of *S. frugiperda*.

Mortality caused by the effect of the water extract of *A. absinthium* on *H. zea* larvae was low compared to other insect pests, reaching only 24% at 8 DAA. However, a study conducted by Brudea (2007) showed that the *A. absinthium* extract at 25 g L⁻¹ water caused 80% mortality of *Aphis spiraeaphaga* Müller at 4 DAA.

The specific insecticidal effect on different Noctuidae pest species must be also considered. For example, Silva et al. (2013) found 75% mortality with the 8% w/v concentration of the *P. boldus* extract on 3rd stadium *S. frugiperda* larvae, while the same concentration caused only 30% mortality on *H. zea* larvae of the same age.

Effect on the number of *H. zea* larvae and damage on fresh ears of corn at harvest. The application of the extracts did not significantly decrease the damage caused by the larvae on the treated ears compared to the untreated control. The interaction of the water extracts of *S. molle* and *A. absinthium* with *Btk* was not significant either. Other plant extracts have also lacked significant efficacy against noctuid pests on corn. For example, a study conducted by Silva (2010) revealed that *P. boldus* extracts did not significantly reduce the damage on leaves by *S. frugiperda* in corn plants under greenhouse conditions

The water leaf extracts from *S. molle* and *Btk*, applied alone or mixed, decreased significantly the number of larvae counted at harvest in the field. However, larval damage did not differ statistically from the control, suggesting that the entry of a minimum larval number is enough to cause severe damage to the ears of corn.

In the tropics, the populations of *H. zea*, itself a tropical origin species, reproduce continuously, and only a small amount of the pupae (2.4%) may enter diapause. In Ecuador, *H. zea* has many

cycles a year and it is widespread throughout the country (Guevara, 2014). This would explain why the insecticidal effect of *Btk* in the field did not differ significantly from the control when the damage on the ears of corn was evaluated.

Heliothis zea is polyphagous, and particularly difficult to control in all farms in counties with small-scale farming. Moreover, larvae prefer plant parts with a high N content, which concentrates on the styles (Guevara, 2014).

In the laboratory, mortality rates at 15 DAA caused by treatments *Btk* 100%, *Btk* 50%, *S. molle* 100% + *Btk* 50%, and *A. absinthium* 50% differed significantly with respect to the control (64, 48, 44, and 40%, respectively). However, this effect did not correlate with the severe damage on the fresh ears of corn caused by the *H. zea* larvae in the field trial as damage level observed in the treatments and untreated control was similar. Thus, other application methods (for extracts and *Btk*) need to be studied considering their volatility under field conditions since corn crops are exposed to different temperatures and even rain.

Sufficient larvae (2.6% on 20 ears) were found causing damage in the field in the treatment with thiamethoxam + lambdacyhalotrin. This indicates the high pressure of the pest, which affects the plants, and also the capacity of some larvae to develop mechanisms of resistance to the chemicals applied. Resistance in *H. zea* to many organo-chlorine, organo-phosphorous, and pyrethroid insecticides have been demonstrated in diverse regions of the world, even up to levels leading to the collapse of agricultural systems (Abd-Elghafar et al., 1993).

The quality and composition of plant extracts is another aspect to consider in the relatively low efficacy exhibited by the extracts evaluated in the lab experiments and field trials. Crude extracts often contain small concentrations of active ingredients and many times they have not been quantified properly. The insecticidal activity of the extract also varies with the climate, soil type, and geographic location of the plant from which it is obtained. The standardization of the product is an aspect that needs to be further studied for most plant extracts intended for use as repellent and/or insecticide against pests (Isman, 2006).

One of the serious challenges to controlling *H. zea* in corn is the rapid entrance of first-instar larvae to the ear of the corn through the fresh styles, where they are safe from the effect of insecticides that act by contact and feeding (Flores, 2010). Because of this, control should be focused on methods that reduce egg laying and subsequent hatching on the styles.

CONCLUSIONS

Only the water extracts from leaves of *S. molle* 100% v/v + *Btk* 50%, and *A. absinthium* 50% v/v caused significant insecticidal effects on 3rd stadium *H. zea* larvae in the laboratory, but no antifeeding effects were observed. In the field, sprays of water extracts from *S. molle* alone or mixed with *Btk* during the fresh style period resulted in a significant decrease in the number of larvae at the harvest of fresh ears of corn, but they did not improve yields. The insecticidal effects observed indicate that these extracts represent new alternatives for the control of *Helicoverpa zea* in maize.

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