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Insecticidal activity of the ethanolic extract from *Croton* species against *Plutella xylostella* L. (Lepidoptera: Plutellidae)

Actividad insecticida de extractos etanólicos de especies de *Croton* contra *Plutella xylostella* L. (Lepidoptera: Plutellidae)

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Keywords:
Croton spp.
Botanical insecticide
Diamondback moth

ABSTRACT

The objective of this work was to study the effect of ethanolic extracts from different parts of *Croton* species on the diamondback moth (*Plutella xylostella* L.). Extracts from the leaves of *Croton rhamnifolius* H.B.K., *Croton jacobinensis* Baill., *Croton sellowii* Baill. and *Croton micans* Muell. *C. rhamnifolius* had the most lethal effect (LC_{50} = 14.95 µg mL^{-1}), followed by *C. rhamnifolius* (stem), *C. jacobinensis* (stem), *C. jacobinensis* (leaf), *C. sellowii* (leaf) and *C. sellowii* (stem) with LC_{50} values of 42.40, 116.21, 183.85, 801.36 and 1252 µg mL^{-1}, respectively. *Plutella xylostella* larvae fed kale disks with all extracts, except *C. sellowii* (stem), exhibited prolonged larval duration. None of the extracts affected the duration of the pupal stage of the moth.

Palabras clave:
Croton spp.
Insecticida botánico
Polilla del repollo

RESUMEN

El objetivo de este trabajo fue estudiar el efecto de los extractos etanólicos de especies de *Croton* en la polilla del repollo (*Plutella xylostella* L.). *Croton rhamnifolius* H.B.K., *Croton jacobinensis* Baill., *Croton sellowii* Baill. y *Croton micans* Muell. *C. rhamnifolius* (hojas) tuvieron el efecto más letal con una CL_{50} de 14,95 µg mL^{-1}, seguido por *C. rhamnifolius* (tallos), *C. jacobinensis* (tallos), *C. jacobinensis* (hojas), *C. sellowii* (hojas) y *C. sellowii* (tallos) con valores de CL_{50} de 42,40; 116,21; 183,85; 801,36 y 1252 µg mL^{-1}, respectivamente. Las larvas de *Plutella xylostella* se alimentaron con discos de col y todos los extractos, excepto *C. sellowii* (tallos), mostraron una duración larval prolongada. Por otra parte, ninguno de los extractos afectó la duración de la etapa de pupas de *P. xylostella*.

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The use of synthetic insecticides is the principal method for controlling *Plutella xylostella* L. (Lepidoptera: Plutellidae) and high doses are often required to minimize the damage caused by this insect. However, populations of this pest exhibit resistance to active ingredients in these insecticides as a result of selective pressure (Gong *et al.,* 2014). Therefore, natural insecticides have been increasingly studied for their effects on the suppression of pest populations, as such substances enable the low-cost control of arthropods, easy management and potential for the discovery of novel insecticidal molecules (Furlong *et al.,* 2013). However, the susceptibility of insects to extracted plant allelochemicals depends on the extraction method, insect species, plant organ and plant species (War *et al.,* 2012).

The mortality rates of *Myzus persicae* Sulzer and second instar *P. xylostella* larvae by extracts from the leaves of *Prosopis juliflora* Swartz was respectively 90 and 28% with the ethanolic extract and 6 and 10% with the aqueous extract (Stein and Klingauf, 1990). Aqueous extracts from *Aspidosperma pyrifolium* Mart, *Azadirachta indica* A. Juss and a commercial formulation made from *A. indica* negatively affected the larval viability of *P. xylostella* (Torres *et al.,* 2001).

Species from the genus *Croton* belong to the family Euphorbiaceae and are found in swamps in the *Caatinga* biome of the state of Pernambuco, Brazil. The species are shrubs or undershrubs that can reach a height of two meters. These plants are often employed in folk remedies as depuratives and agents for relieving pain, vomiting and bloody diarrhea (Braga, 1976). According to the communities around the collection sites, *Croton rhamnifolius* H.B.K., *C. jacobinensis* Baill., *C. sellowii* Baill. and *C. micans* Muell. are considered to be useful for gastric ailments, stomach pain and attenuating fever.

Croton species are known for the production and accumulation of terpenoids, especially monoterpenes, sesquiterpenes and diterpenes, which are generally found in all parts of the plant and with considerable structural diversity, including many bioactive molecules with effects against arthropods (Filho *et al.,* 2013; Kuo *et al.,* 2013).

As part of a systematic study on the insecticidal potential of the medicinal flora growing in the state of Pernambuco (Bandeira *et al.,* 2013; Pereira *et al.,* 2009), the objective of this work was to evaluate the potential of ethanolic extracts prepared from the stems and leaves of *C. rhamnifolius*, *C. jacobinensis*, *C. sellowii* and *C. micans*. on the development and survival of *P. xylostella*. This is the first report of the insecticidal action of organic extracts from species of *Croton*.

MATERIALS AND METHODS

Plant material

The leaves and stems of the plants investigated were collected during the morning hours. *C. jacobinensis* Baill. (08°08'39"S 36°22'17"W; altitude: 1800 m), *C. micans* Muell. (08°08'17"S 36°21'52"W; altitude: 900 m), *C. rhamnifolius* H.B.K (08°08'13"S 36°21'47"W; altitude: 795 m) and *C. sellowii* Baill (08°21'07"S 34°56'34"W; altitude: 300 m) were selected for the bioassays. The former three species of *Croton* were collected from highland forests in the municipality of Brejo da Madre de Deus and the latter species was collected from a fragment of the Atlantic forest in the municipality of Cabo de Santo Agostinho, state of Pernambuco, Brazil. The plants were identified by botanist Dr. Maria de Fátima Araújo Lucena, researcher from the *Universidade Federal de Pernambuco*. Voucher specimens of each species were registered under the numbers 45553 (*C. jacobinensis*), 48218 (*C. micans*), 48217 (*C. rhamnifolius*) and 45622 (*C. sellowii*) and maintained at the "Vasconcelos Sobrinho" Herbarium of the Botany Department, *Universidade Federal Rural de Pernambuco* (UFRPE), Brazil.

Organic extract preparations

For the preparation of the extracts, leaves and stems were washed and oven-dried at 40 °C for 48 h. Portions of each species were ground in a mill and weighed separately. Stems and leaves were placed in separate flasks and ethanol was added until covering all the plant material. Maceration was performed at 24-h intervals for 72 h to ensure the complete extraction of all active substances. The extract was filtered and evaporated at low pressure to minimize possible degradation of the chemical constituents at high temperatures. After removal of the solvent, the crude ethanol extracts of the plants were obtained. Yield was expressed as

a percentage value (g per 100 g of dry weight plant material).

The concentrations used were prepared from stock solutions of the different ethanolic extracts. A one-gram aliquot of ethanol extract was suspended in 19.9 mL of distilled water and 0.1 mL of Tween 80 as dispersant. The solution was agitated until the complete dissolution of the extract and filtered through a Whatman No. 1 paper filter for the obtainment of the stock solution (50 mg mL^{-1}) for the preparation of the different concentrations used in the bioassays. Dilutions of the aqueous stock solution were used for the immersion solution (50 mL) at the desired concentrations, ranging from 5.0 µg mL^{-1} to 4000 µg mL^{-1}.

Insect and general procedures of experiments

Rearing of *P. xylostella* was performed from pupae maintained at the UFRPE Laboratory of Insect Biology. Newly-emerged adults were sexed and placed in plastic cages with a sponge soaked in water to maintain proper humidity. A disk of filter paper (Ø 8 cm) and a leaf disk of kale (*Brassica oleracea* L. var. *acephala*) were placed on the sponge to stimulate oviposition. The adults were fed a 10% honey solution provided in a polyurethane foam recipient attached to a circular hole at the top of the cage.

Bioassay

Kale leaf disks with the eggs were transferred to Petri dishes daily and kept until the hatching of the *P. xylostella*. Disks containing the larvae were kept in rectangular plastic containers with organic kale leaves, which were used as food. The larvae remained in these containers and the kale leaves were changed daily until the larvae reached the pupal stage.

Pupae were collected in test tubes covered with PVC film that enabled the circulation of air and kept at room temperature until the emergence of the adults, which were transferred to cages. Kale leaf disks measuring 8 cm in diameter were sprayed with 2 mL of a water-alcohol solution and different concentrations of crude ethanolic extracts from the stems and leaves of the *Croton* species in a Potter spray tower calibrated to a pressure of 10 psi. Kale leaf disks sprayed with the water-alcohol solution without the extracts were used as control. After spraying, the disks were placed on filter paper at room temperature to remove excess moisture, subsequently transferred to Petri dishes and placed on filter paper disks. The tests were conducted at a temperature of 30 ± 1 °C, 70 ± 10% relative humidity and a 12-h photoperiod. Preliminary tests were performed to determine extract concentration ranges that caused insect mortality from near zero to near 100%. More precise response ranges for average lethal concentrations (LC_{50}) were obtained from this wide range.

The method for evaluating the larval and pupal viability after exposure to different concentrations of crude ethanolic extracts was outlined by Torres *et al.* (2001) and Boiça-Junior *et al.* (2005). Treated leaf disks were placed in Petri dishes containing filter paper moistened with distilled water. Ten newly-hatched *P. xylostella* larvae were confined in each dish. The number of individuals dead was determined 72 h after the confinement of the larvae and treated kale disks were replaced with untreated leaves. Other assessments were performed every 24 h and leaf disks were changed every 48 h until the larvae reached the pupal stage. The pupae from each treatment were isolated on ELISA plates and the viability assessment was performed by daily monitoring for the emergence of adults.

Statistical analyses

The experimental design was completely randomized, consisting of 47 treatments (concentrations of the extracts from the stems and leaves of *Croton* species) with 10 replicates, each replicate containing 10 newly-hatched *P. xylostella* larvae. Extract concentration-larval mortality curves were calculated using the Statistical Analysis Software (SAS Institute, 2002). Data were also analyzed with the Probit model with the aid of the Polo-PC software program (LeOra Software, 1987) for the determination of the LC_{50} (lethal concentration for 50% population mortality) with 95 percent confidence levels for all experiments. Some concentrations were suppressed to better fit the model for the calculation of LC_{50}. Mortality data for extracts were fitted to the Probit model (χ^2 test; $P>0.05$). The results were submitted to a regression analysis to detect whether the extracts influenced larval and pupal duration (SAS Institute, 2002).

RESULTS AND DISCUSSION

The yield of the extracts varied depending on the part of the plant and species studied. However, yields were slightly higher for the leaf extracts in comparison to the stem extracts, regardless of the species investigated (Table 1).

Table 1. Percentage of crude ethanolic extracts from stems and leaves of species of *Croton.*

Species	Plant part	Quantity (g)	%
C. rhaminifolius	stem	250	3.63
	leaf	250	5.57
C. jacobinensis	stem	220	4.42
	leaf	300	5.90
C. sellowii	stem	230	3.25
	leaf	300	5.01
C. micans	leaf	270	4.50

All extracts tested were toxic to *P. xylostella* larvae. Toxicity also varied depending on the part of the plant and species. The leaf extract from *C. rhamnifolius* demonstrated the greatest toxicity, as demonstrated by the the lowest concentration required for 50% population mortality (LC_{50} = 14.95 µg mL^{-1}), followed by extracts from *C. rhamnifolius* (stem), *C. jacobinensis* (stem), *C. jacobinensis* (leaf), *C. sellowii* (leaf), *C. sellowii* (stem), with LC_{50} values of 42.4, 116.21, 183.85, 801.36 and 1252 µg mL^{-1}, respectively. The leaf extract from *C. micans* did not demonstrate Probit distribution due to the heterogeneity of the data (Table 2).

The lethal concentrations of *C. rhamnifolius* (leaf), *C. rhamnifolius* (stem), *C. jacobinensis* (leaf) and *C. sellowii* (leaf) were similar. Extracts from the stems of

Table 2. Toxicity of ethanolic extracts from species of *Croton* to first-instar *P. xylostella* larvae fed on leaves of *B. oleracea* var. *acephala.*

Extract		n	Equation (95% CI)	LC_{50} (µg mL^{-1}) (95% CI)	χ^2	*P*	TR
RHAM	Leaf	600	$Y = -1.49 + 1.27\log_{conc.}$ (1.06 – 1.48) b	14.95 e (11.03 – 19.22)	3.44	0.4858	-
	Stem	400	$Y = -2.14 + 1.31\log_{conc.}$ (1.08 – 1.55) b	42.40 d (32.89 – 55.56)	3.78	0.1510	2.83
JACO	Leaf	600	$Y = -2.89 + 1.28\log_{conc.}$ (1.07 – 1.49) b	183.85 c (148.47 –224.63)	6.80	0.1468	12.30
	Stem	600	$Y = -1.53 + 0.74\log_{conc.}$ (0.61 – 0.88) c	116.21 c (78.30 –164.15)	6.86	0.1432	10.78
SELL	Leaf	500	$Y = -4.85 + 1.67\log_{conc.}$ (1.38 – 1.97) b	801.36 b (670.23 – 951.21)	2.91	0.4062	53.60
	Stem	400	$Y = -8.14 + 2.63_{conc.}$ (2.211 – 3.045) a	1252.00 a (1064.00 –1446.00)	0.21	0.8990	83.75
MICA	Leaf	700		No PROBIT			

RHAM = *C. rhamnifolius*; JACO = *C. jacobinensis*; SELL = *C. sellowii*; MICA = *C. micans*; n = number of insects tested; CI = confidence interval for 95% probability for slope; χ^2 = chi-squared; TR = toxicity ratio; Equation and LC_{50} value followed by same letter in column do not differ significantly (*P*>0.05, Tukey's test).

C. jacobinensis and *C. sellowii* differed between each other as well as the other treatments.

The steepest slope of the mortality curve for *P. xylostella* occurred with the extract from *C. sellowii* (leaf), signifying that small variations the concentration of this extract can cause large variations in its mortality potential. The toxicity ratio between the leaf extract from *C. rhamnifolius* and extracts from *C. rhamnifolius* (stem), *C. jacobinensis* (leaf), *C. jacobinensis* (stem), *C. sellowii* (leaf) and *C. sellowii* (stem) was 2.83, 12.30, 10.78, 53.60 and 83.75, respectively (Table 2).

Comparing the present results with those reported in the literature for the same pest involving extracts prepared with different solvents, the *C. rhamnifolius* extract is much more toxic. For example, Boiça Junior *et al.* (2005) used greater amounts of *Enterolobium contortisillidium* (fruit), *Nicotiana tabacum* (leaf), *Sapindus saponaria* (fruit) and *Trichilia pallida* (twigs) to achieve a 100% mortality rate of *P. xylostella* larvae fed on kale (*B. oleracea* var. *acephala*) treated with 10% aqueous extracts. Torres *et al.* (2001) report the same result with 10% aqueous extracts from the seeds and bark of *Azadirachta indica* A. Juss. and *Aspidosperma pyrifolium* Mart.. Li *et al.* (2008) found that the acetone fraction of the chloroform extract from *Xanhtium sibiricum* promoted a 91.67% larval mortality rate at a concentration of 50 µg mL^{-1}, whereas the ethanolic extract from *C. rhamnifolius* in the present investigation had an LC_{50} of 14.95 µg mL^{-1}. *Rani et al.* (1999) achieved a 100% mortality rate of *P. xylostella* larvae with an ethanol extract from the twigs of *Melia azaderach* at a concentration greater of 7.5%, which is higher than that used in the present study.

On the other hand, using chemical constituents isolated from active extracts, Yang *et al.* (2008) reported insecticidal action against *P. xylostella* larvae for two active chemical components from the fruit of *Ginkgo biloba* L., with estimated LC_{50} values for bilobol (LC_{50} = 2.0613 g L^{-1}) and ginkgo acid (LC_{50} = 4.6002 g L^{-1}) that were respectively 3.3-fold and 7.3-fold greater than estimated LC_{50} for the leaf extract from *C. rhamnifolius*, which achieved the best result in the present study. Moreover, the ethanolic extract from *Zanthoxylum armatum* (Kumar *et al.,* 2016) was slightly more toxic than the ethanolic leaf extract from *C. rhamnifolius*. However, in comparison to oil from the fruit of *Azadirachta indica* (Kolani *et al.,* 2016), the leaf extract from *C. rhamnifolius* was about 3.9-fold more toxic.

With regard to the viability of *P. xylostella* larvae and pupae exposed to *Croton* extracts, only very high concentrations of the stem extracts from *C. jacobinensis* and *C. rhamnifolius* and the leaf extracts from *C. sellowii* and *C. rhamnifolius* caused 100% mortality (Table 3).

Table 3. Larval and pupal viability based on larval mortality and total mortality (larvae and pupae of *Plutella xylostella* larvae (Lepidoptera: Plutellidae) fed leaf disks of *B. oleracea* var. *acephala* treated with different concentrations of ethanolic extracts from species of *Croton.*

Species	Part of plant	Concentration (µg mL^{-1})	Larval mortality	Total mortality
C. rhamnifolius	Leaf	5	24.0 ± 0.26	40.0 ± 4.22
		25	67.0 ± 0.44	77.0 ± 3.67
		50	77.0 ± 0.47	85.0 ± 3.73
		100	82.0 ± 0.48	87.0 ± 3.35
		200	91.0 ± 0.51	95.0 ± 1.67
		400	97.0 ± 0.53	100.0 ± 0.00
	Stem	5	15.0 ± 0.21	32.0 ± 3.89
		25	32.0 ± 0.31	44.0 ± 10.24
		50	53.0 ± 0.39	59.0 ± 5.26
		100	48.0 ± 0.37	72.0 ± 5.54
		200	57.0 ± 0.40	74.0 ± 5.62
		400	67.0 ± 0.42	75.0 ± 6.19
		800	84.0 ± 0.51	100.0 ± 0.00
		4000	93.0 ± 0.52	100.0 ± 0.00

Table 3. continuation

Species	Part of plant	Concentration (µg mL^{-1})	Larval mortality	Total mortality
C. jacobinensis	Leaf	25	18.0 ± 0.23	32.0 ± 4.67
		50	30.0 ± 0.30	39.0 ± 3.79
		100	35.0 ± 0.32	46.0 ± 3.40
		200	45.0 ± 0.36	55.0 ± 2.69
		400	62.0 ± 0.43	71.0 ± 5.67
		800	84.0 ± 0.49	88.0 ± 3.60
		1600	91.0 ± 0.51	95.0 ± 3.07
	Stem	25	32.0 ± 0.29	40.0 ± 7.88
		50	45.0 ± 0.37	55.0 ± 8.72
		100	48.0 ± 0.37	53.0 ± 5.78
		500	47.0 ± 037	61.0 ± 7.29
		1000	70.0 ± 0.45	82.0 ± 6.63
		2000	79.0 ± 0.48	100.0 ± 0.00
		4000	93.0 ± 0.52	100.0 ± 0.00
C. sellowii	Leaf	250	17.0 ± 023	30.0 ± 6.15
		500	41.0 ± 0.34	65.0 ± 4.54
		1000	59.0 ± 0.41	75.0 ± 4.28
		2000	70.0 ± 0.52	100.0 ± 0.00
		4000	74.0 ± 0.46	100.0 ± 0.00
		6000	82.0 ± 0.48	100.0 ± 0.00
		8000	96.0 ± 0.52	100.0 ± 0.00
	Stem	250	7.0 ± 0.14	32.0 ± 5.13
		500	15.0 ± 0.21	27.0 ± 5.18
		1000	23.0 ± 0.24	75.0 ± 5.82
		2000	67.0 ± 0.45	90.0 ± 4.30
		4000	90.0 ± 0.51	96.0 ± 1.63
		6000	97.0 ± 0.53	98.0 ± 1.33
C. micans	Leaf	250	12.0 ± 0.19	230 ± 4.23
		500	16.0 ± 0.22	260 ± 4.27
		1000	15.0 ± 0.21	27.0 ± 4.95
		2000	21.0 ± 0.25	33.0 ± 7.62
		4000	40.0 ± 0.34	53.0 ± 4.48
		6000	73.0 ± 0.46	88.0 ± 5.12
		8000	93.0 ± 0.52	95.0 ± 2.69

P. xylostella larvae fed on *B. oleracea* var. *acephala* disks treated with leaf extracts from *C. jacobinensis*, *C. rhamnifolius*, *C. sellowii* and *C. micans* and stem extracts from *C. jacobinensis* and *C. rhamnifolius* exhibited prolonged duration of the larval stage (Figure 1). For the stem extract from *C. sellowii,* the Y value of the equation averaged 7.2 ± 0.06 days, demonstrating no significant effect.

Based on these data, we can infer that the leaf extract of *C. rhamnifolius* was the most toxic to *P. xylostella*. This result may be associated with its higher concentrations of deterrents, as found in *Croton jatrophoides*, which contains limonoids with anti-feeding properties for *Pectinophora gossypiella* Sauders (Lepidoptera: Gelechiidae) and *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Nihei *et al.,* 2006). Moreover, species of *Croton* contain higher concentrations of terpenoids in leaves and roots, which probably resulted in the higher *P. xylostella* mortality found for the leaf extract from *C. rhamnifolius* (Randau *et al.,* 2004).

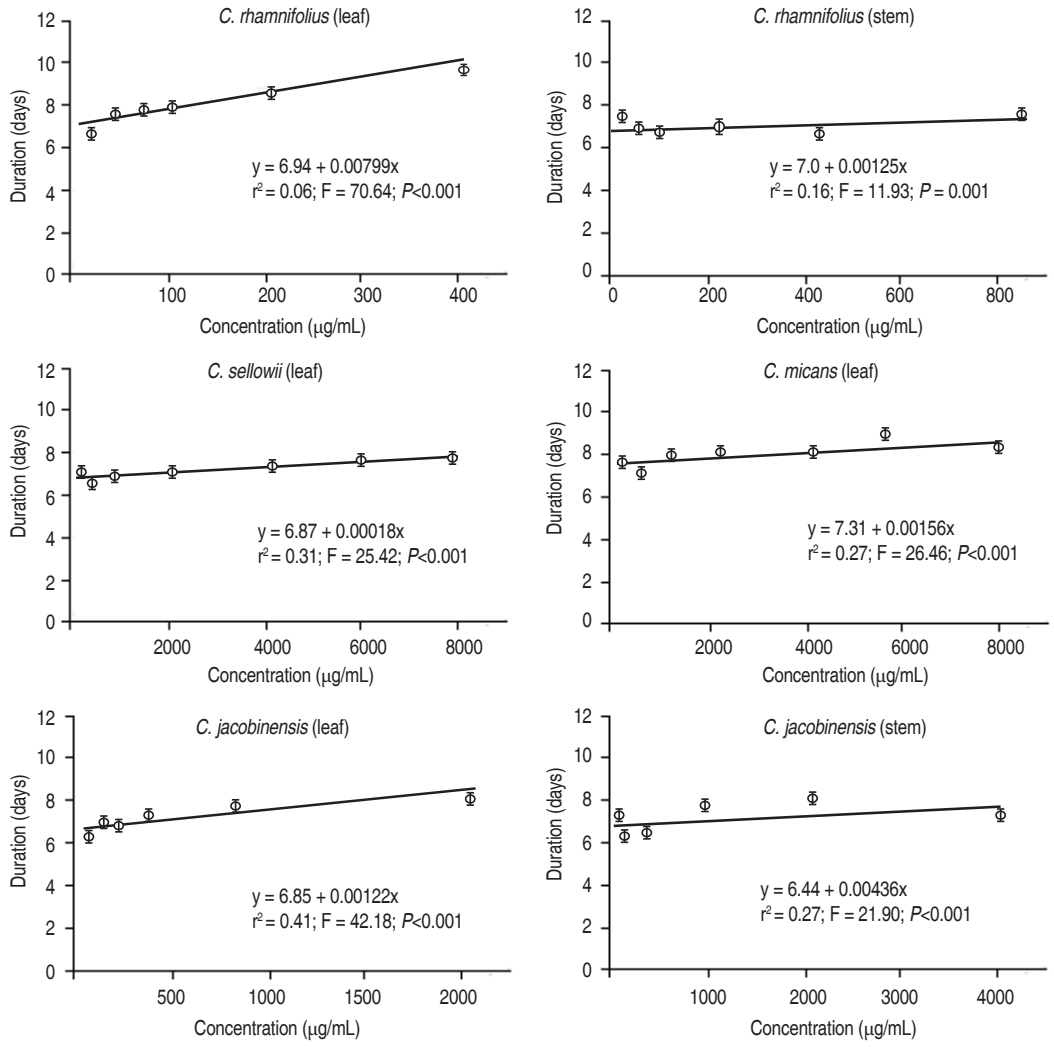

Figure 1. Effect of extracts from species of *Croton* on duration of larval stage of *Plutella xylostella*.

Extract activity is associated with the part of the plant from which the extraction is taken (Trindade *et al.,* 2000), as demonstrated by the greater toxicity of the leaf extracts from *C. rhamnifolius* and *C. sellowii* compared to the stem extracts of these plants. Ethanolic extracts from the stems, bark, roots and fruit of *A. pyrifolium* exhibited differences in *P. xylostella* mortality, which is similar to the findings for *C. rhamnifolius* in the present study (Trindade *et al.,* 2008).

All extracts, except the leaf extract from *C. rhamnifolius*, prolonged the larval phase (Figure 2). In practical terms, this result is quite important, as it increases the average time for each generation of the insect, consequently increasing the exposure to natural enemies. In contrast, no extract affected the duration of the pupal phase, although all extracts affected pupal viability (Table 3).

Extract activity on *P. xylostella* reflects the diversity of secondary metabolites produced by plants of the genus *Croton* (Marino *et al.,* 2008). On other hand, insecticidal activity is associated with the solvent, which can make an extract more efficient depending on the insect tested (Moreira *et al.,* 2007).

The longer cycle of *P. xylostella* with ethanol extracts from the leaves of *C. jacobinensis*, *C. rhamnifolius*,

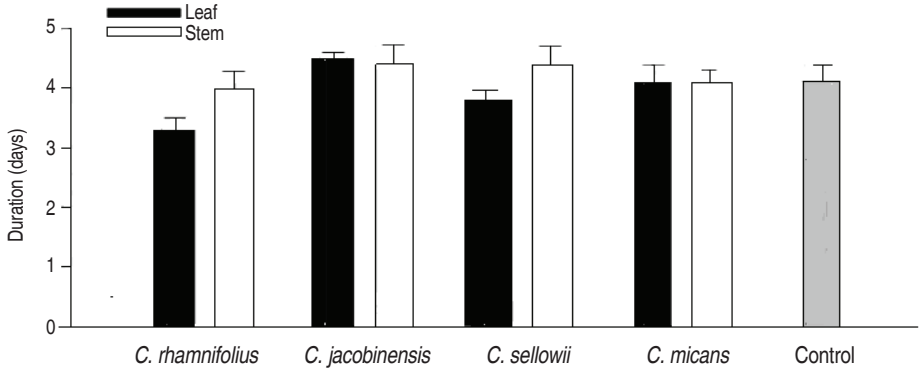

Figure 2. Effect of extracts from species of *Croton* on duration of pupal stage of *Plutella xylostella*.

C. sellowii, *C. micans* and stems of *C. jacobinensis* and *C. rhamnifolius* suggests that these plants have inhibitory properties that affect the development of this pest. The present findings suggest that *Croton* plants have substances with an insecticidal effect against *P. xylostella*. Thus, investigation of these resources could provide new substances for the control of the diamondback moth. All ethanolic extracts from the species of *Croton* evaluated were toxic to *P. xylostella* in the larval phase, with greatest toxicity found for the ethanolic leaf extract from *Croton rhamnifolius*. On the other hand, none of the extracts affected the duration of the pupal stage, which indicates an effect on food intake in *P. xylostella*.

CONCLUSIONS

The results demonstrate that the search for insecticidal properties in *Croton* plants is promising with regard to the discovery of new vegetal species for the control of agricultural pests. With the exception of the raw extract from the stems of *C. micans*, all extracts were active and caused 100% mortality of larvae and pupas at the highest concentrations. The greatest toxicity to larvae was found with the leaf extract from *C. rhamnifolius*, which caused 100% mortality at a concentration of 400 µg mL^{-1} one day after treatment, with an estimated LC_{50} of 14.95 µg mL^{-1}. However, considering the angular coefficients (ß) of the equations obtained regarding the toxicity of the different raw extracts tested, the greatest slope of the curve occurred for the leaf extract from *C. sellowii*, which means that small variations in the amount of this extract can cause a considerable change in its insecticidal potential. The present results reveal the insecticidal potential of the crude ethanolic extract from the leaves of *C. rhamnifolius*. Bio-monitored fractioning of this extract to isolate and identify the active ingredient and its possible mechanism of action is under way.

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