Effectiveness of Commercial Plant Extracts in Management of *Plutella xylostella* (Lepidoptera: Plutellidae) on Broccoli in Mexico[1]

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Abstract *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is the primary pest of broccoli (*Brassica oleracea* var. *italica* von Plenck), and it holds the distinction of having the highest number of documented cases of insecticide resistance among insect pests, which complicates its management. The utilization of plant extracts is considered an alternative approach. Therefore, this study aimed to determine the efficacy of commercial plant extracts for the control of *P. xylostella*. Nine commercial extract products were evaluated, and their effects were compared with Inex-A® + distilled water (control group) and Exalt® (commercial control). Toxicity assays were conducted using third-instar larvae by topical application bioassay. Oviposition inhibition was assessed along with adult female repellency by exposing females to treated surfaces. To determine the residual effect on feeding and oviposition inhibition, third-instar larvae and 3- to 6-d-old adult females were used, respectively, with residual effects measured at 2, 4, 6, 8, and 10 d postapplication. Nimicide 80 (Ultraquimia, Morelos, Mexico) exhibited a mortality rate of 35% of third-instar larvae. Garlimax (Plant Health Care, Azcapotzalco, Mexico) inhibited oviposition by 80%, and demonstrated 85% repellency. The residual effect of Garlimax persisted for 4 d, resulting in a reduced percentage of the consumed area and egg oviposition. The findings of this research highlight the effectiveness of these two products, Nimicide 80 and Garlimax; however, field evaluations are recommended to verify the consistency of their biological effects.

Key Words toxicity, repellency, residual activity, inhibition, plant extracts

Broccoli (*Brassica oleracea* var. *italica* von Plenck) is one of the most significant crops in Mexico, with an annual production in 2022 of 632,258 tons, of which 71.8% was exported, primarily to the United States. The state of Guanajuato contributes 65% of this total production (SIAP 2023). However, cultivation and yield of broccoli crops is overwhelmingly affected by larvae of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae).

Plutella xylostella is a pest native to the Mediterranean, a region that also serves as the center of origin for the most important species of the Brassicaceae family.

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This pest is distributed in all countries where broccoli, cabbage, and cauliflower are cultivated (Bujanos et al. 2013). In its larval stage, it causes damage to the foliage, hinders plant growth, and can lead to the death of the plant (Da Silva 2008). The presence of larvae, pupae, or their residues diminishes the commercial potential of the final product (Montero et al. 2007, Vásquez et al. 2008). Even before floret formation, high infestations of *P. xylostella* can cause deformation and reduce plant weight (Grzywacz et al. 2010). The global economic losses caused by diamondback moth amount to approximately US$168 million/yr (Uthamasamy et al. 2011).

The diamondback moth is considered one of the most challenging pests to control, and the most common methods are the application of insecticides such as diamide, avermectin, pyrethrin, and *Bacillus thuringiensis* Berliner groups (Xia et al. 2014). The overuse of these products has led to the selection of resistant populations (Santos et al. 2011). According to the Arthropod Pesticide Resistance Database (APRD 2022), 1,022 cases of resistance to various chemical groups of insecticides have been reported for *P. xylostella*. It is, therefore, essential to establish a foundation for an integrated pest management strategy, including the use of plant extracts, which have shown promising results. For instance, the extract of *Calotropis gigantea* L. (Asclepiadaceae) has demonstrated toxic and antifeedant effects on *P. xylostella* larvae (Khasanah et al. 2021). Applying extracts of *Parthenium hysterophorus* L. (Asteraceae) and *Lantana camara* L. (Verbenaceae) resulted in toxic effects and repellency against adult *P. xylostella* (Reddy et al. 2018, Thanavendanand and Kennedy 2015). Garlic, rosewood, and thyme extracts have been documented to inhibit oviposition and feeding, as well as exhibit toxic effects on *P. xylostella* larvae and adults (Sangha et al. 2017). The application of neem extract (Neemix®) also has shown inhibition of oviposition and feeding in *P. xylostella* (Liu and Liu 2006), whereas the use of garlic and chili extracts reduces foliar damage by diamondback moth larvae because of the presence of triterpenoid saponins acting as feeding deterrents (Lamba and Malapa 2020).

Hence, it is crucial to assess the effects of commercially available plant extracts that may have potential to control *P. xylostella*. The objective of this research was to characterize the toxicity, oviposition and feeding inhibition, repellency, and residual activity of several commercial plant extracts on *P. xylostella*.

Materials and Methods

Insects, plants, and extracts. A colony of *P. xylostella* was established in the Biological Control Laboratory at Colegio de Postgraduados, Montecillo Campus, Texcoco, state of Mexico. The colony originated from various municipalities in Guanajuato, Mexico and was maintained in a bioclimatic chamber (25°C $\pm$ 2°C, 75% $\pm$ 5% relative humidity [RH], and 12:12 h light:dark [L:D]). Adult diamondback moths were housed in organza frame cages (30 $\times$ 60 $\times$ 30 cm), 40 males and 40 females in each. Their diet was comprised of honey droplets in a Petri dish and a cotton wick with water. Broccoli plants (35 d old) were introduced into these cages for 48 h to facilitate oviposition. Plants with eggs were then labeled with the oviposition date and placed in a plastic container to monitor the age of the pest. Larvae developed on broccoli plants, which were replaced as needed, and pupae were collected and placed in a Petri dish within the adult cage.

Broccoli plants used for *P. xylostella* rearing were planted in 1-L pots with a peat moss and tezontle mixture in a greenhouse at Colegio de Postgraduados, Montecillo Campus. These plants were fertilized with a 25% Steiner nutrient solution and irrigated using an automated drip irrigation system.

Nine commercial insecticide plant extracts, the commercial control Exalt® (Spinetoram J + Spinetoram L), and an absolute control (Inex®-A surfactant + distilled water) were evaluated in this study (Table 1). Each plant extract was tested at the manufacturer's recommended concentration, using the specified water volume indicated on the label.

Toxicity bioassay. For this topical application bioassay, the methodology of Morales et al. (2015) and Ramírez-Cerón et al. (2022) was used with slight modifications. Ten third-instar *P. xylostella* larvae were anesthetized with CO_2 for 10 s and placed on filter paper in a glass Petri dish (15-cm diameter). Treatments were applied using an acrylic Potter tower (0.5-cm thickness measuring 150 cm high, 50 cm wide, and 50 cm long). It featured a solid cone nebulization nozzle (cat.1/4 J-SS + SU1A-SS, Spraying Systems, Wheaton, IL) connected to a constant air pressure source. The system was calibrated to apply 2 mg/cm^2 of the extract using a 3-mL solution (extract + distilled water + Inex-A surfactant) at 20 psi pressure. The application was made 120 cm from the nebulization source.

Subsequently, treated larvae were placed on a broccoli leaf disc arranged on an agar–water base (0.5-cm thickness) (ratio 1.6 g:100 mL) in a Petri dish, with the lid having a 1.0-cm-diameter hole covered with organza fabric for ventilation. These experimental arenas were maintained under controlled conditions (25°C ± 2°C, 75% ± 5% RH, and 12:12 h L:D). Larval mortality was recorded at 24, 48, and 72 h postapplication. To confirm their status, larvae were stimulated with a No. 000 brush, and if no movement was observed, they were considered dead. Ten replications consisting of 10 *P. xylostella* larvae each were conducted per treatment.

Oviposition inhibition and repellency in adult *P. xylostella*. For this assay, the experimental arena was a fabric cage (30 × 20 × 20 cm) and four broccoli leaf discs (two from the upper side and two from the lower side). These discs were placed on an agar–water base (0.30-cm thickness) (ratio 1.6 g:100 mL) in a Petri dish lid (5-cm diameter). The lids containing the leaf discs were vertically positioned on the back wall of the cage using Velcro. Of the four discs in the experimental arena, two were sprayed with each treatment (1 L of distilled water was used to dilute the dose of each extract) and the other two were sprayed with the absolute control. Five mated females ages 3–6 d were released within the cage; the number of eggs deposited on each disc was recorded 24 h later.

To assess the repellency of adult diamondback moths to treatments, a miniature Anbolm camera with 365° night vision (model ML8-133, Anbolm; Hikvision, Hangzhou, China,) was placed inside and in the center of the bottom of the cage, capturing footage from 7:00 p.m. onward because of the pest's crepuscular habits. Repellency was evaluated for 10 min at 0.5, 1, 2, 3, and 4 h postapplication, recording the number of times moths landed on the treated broccoli leaf surfaces (upper and lower sides). Ten replications per treatment were conducted on different dates.

Residual activity on *P. xylostella* feeding. Two-month-old broccoli plants were used for this assay, each of which was sprayed with the plant extract from each treatment using a manual sprayer with a hollow cone nozzle (22 psi). To

Table 1. Products and application rates for the control of *Plutella xylostella* larvae and adults with commercially available plant extracts.

Treatment No.	Commercial Name	Company	Active Ingredient and Concentration	Application Rate
T1	Bio Crack®	BerniLabs	Garlic (87 %), chamomile, and rue (10 %)	2.0 L/ha
T2	Garlimax®	Plant Health Care México	Garlic (25 %)	2.5 L/ha
T3	Bio Capsi Xtra®	Bio Nutra	Chili, cinnamon, castor, and garlic (34.4 %)	1.5 L/ha
T4	Biodi®E	Ultraquimia	Argemonin (3.5%), berberine (2.2%), ricinine (2.8%), and a-terthienil (3.5%)	3 L/ha
T5	Nimicide 80®	Ultraquimia	Neem (80 %)	3 L/ha
T6	Biotika®Ricinus	Biorganix	Castor (90 %)	7.5 mL/L
T7	Organoil Plus®	Bio Nutra	Sunflower, oregano, and garlic (50.4 %)	1.5 L/ha
T8	CinnAcar®	Ultraquimia	Cinnamon (15 %)	1.75 L/ha
T9	Natural King®	Ultraquimia	Wormseed (60 %)	1.5 L/ha
T10	Exalt®	Corteva Agriscience	Spinetoram J + Spinetoram L (60 %)	0.25 L/ha
T11	Absolute control	—	Inex-A® + distilled water	1 mL/L

dilute the dose of each extract, 1 L of distilled water was used. Each plant was considered a replicate. The plants were placed on a wooden base outdoors and covered at the top with plastic solely to prevent rain entry. Later, one leaf was taken from each sprayed plant every 48 h (2, 4, 6, 8, and 10 d postapplication). A 5.0-cm-diameter leaf disc was cut from this leaf and placed on an agar–water base (0.5-cm thickness) (ratio 1.6 g:100 mL) in a Petri dish of the same dimensions. Subsequently, the leaf disc was exposed to the feeding of five third-instar *P. xylostella* larvae. At 48 h after exposure, a photograph of the leaf disc was taken using a Canon Rebel T6i camera with an 18–55-mm zoom lens (Canon, Tokyo, Japan). The image was segmented using the GIMP© program (Ver. 2.10.36, 2023-11-05; https:/www.gimp.org) and, finally, the percentage of consumed area was determined using the Image J program (Ver. 1.53t) (Rasband 1997– 2018). Eight replications per treatment with five leaves per replicate were performed.

Residual activity in inhibition of *P. xylostella* oviposition. For this assay, a methodology similar to the feeding inhibition residuality test described above was used. Broccoli plants were sprayed with one of the treatments (1 L of distilled water was used to dilute the dose of each extract) and placed on a wooden base, covered with plastic on top to prevent rain entry. At 48 h after treatment application (2, 4, 6, 8, and 10 d), a broccoli leaf was removed and placed in a container with water to maintain turgidity. Each leaf was placed in a frame cage ($30 \times 20 \times 20$ cm, one cage per treatment) where five mated *P. xylostella* females ages 3–6 d were released. The number of eggs oviposited on each leaf was counted 24 h after exposure. Ten replications per treatment were conducted, each with five leaves per replicate.

Statistical analysis. Larval toxicity data were analyzed using a nonparametric Kruskal–Wallis analysis with Bonferroni mean comparison. A chi-square independence test was performed to obtain data from the oviposition inhibition and repellency experiment. Regarding data from the residuality experiment on feeding and oviposition inhibition, an analysis of variance and a mean separation test using the least significant difference method ($\alpha = 0.05$) were conducted. The assumption of variance homogeneity was validated using the Levene method, and data normality was assessed through the Shapiro–Wilk test. All statistical analyses were performed using the R statistical program with the Agricolae library (R Development Core Team 2020).

Results

Toxicity bioassay. Significant differences were observed in toxicity to third-instar larvae at 24 h ($\chi^2 = 52.74$, df $= 10$, $P < 0.0001$). The plant extracts yielded mortality levels ranging from 0% to 9.0%, whereas the commercial control registered 79.0% mortality. At 48 h, significant differences among treatments were observed ($\chi^2 = 62.99$, df $= 10$, $P < 0.0001$). The commercial control caused 88.0% mortality, and Bio Crack, Garlimax, Bio Capsi, Biodi E, and Nimicide 80 products yielded mortality values ranging from 11.0% to 18.0%. The remaining products (Biotika Ricinus, Organoil Plus, CinnAcar, and Natural King) caused mortality below 6.0%. At 72 h, significant differences among treatments were again detected ($\chi^2 = 70.25$, df $= 10$, $P < 0.0001$). The commercial control achieved a mortality exceeding 92.0%, followed by the plant extract Nimicide 80, with a

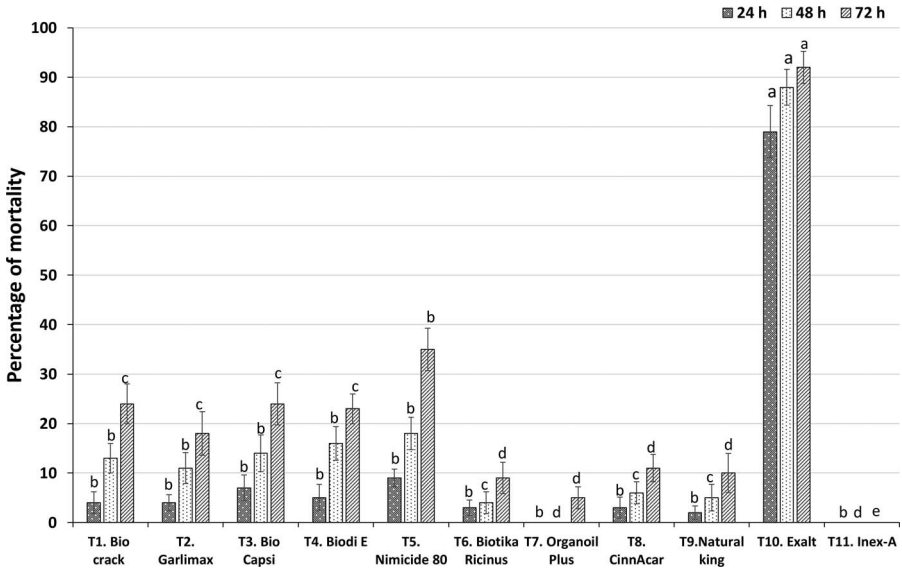

Fig. 1. Mortality of third-instar *Plutella xylostella* larvae at 24, 48, and 72 h after application of commercial plant extracts. Bars of the same shape with identical letters are not statistically different ($P \leq 0.05$).

mortality of 35.0%. The remainder of the products caused mortality ranging from 5.0% to 24.0%. The Inex-A did not induce mortality (Fig. 1).

Oviposition inhibition and repellency effect in adult *P. xylostella*. Significant differences in percent oviposition were observed among the treatments compared with leaves treated with the absolute control. The Garlimax treatment ($\chi^2 = 101.97$, df $= 9$, $P < 0.0001$) showed the highest inhibition of oviposition, registering only 18.4% of the total eggs laid, whereas leaves treated with the absolute control recorded 81.6% of deposited eggs. Other treatments, such as Organoil Plus and CinnAcar, supported 28.0% and 27.9% of eggs oviposited, respectively, compared with their absolute controls, which obtained 71.9% and 72.0% oviposition. The remaining treatments (Bio Crack, Bio Capsi, Biodi E, Nimicide 80, Biotika Ricinus, Natural King, and Exalt) recorded 32.8–43.8% of eggs oviposited on leaves treated with plant extracts, whereas leaves treated with absolute controls had 56.1–67.1% oviposition (Fig. 2).

In repellency trials, all applied treatments caused an effect that limited the number of times diamondback moth adults landed on the leaf discs compared with their respective controls. The product Garlimax reported the highest repellency compared with its respective control ($\chi^2 = 220.61$, df $= 9$, $P < 0.0001$). Of the total adult landings on leaves, only 12.5% *P. xylostella* adults landed on leaves treated with Garlimax, compared with its absolute control in which 87.4% of adults landed. The product Nimicide 80 exhibited a significant repellent effect ($\chi^2 = 220.19$, df $= 9$, $P < 0.0001$), with 16.3% of recorded instances of adults landing, whereas with the absolute control 83.9% landed (Table 2).

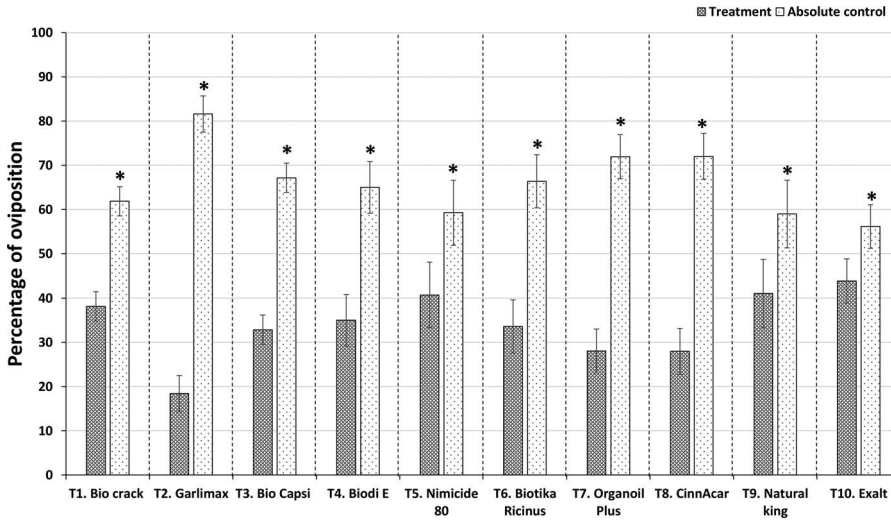

Fig. 2. Percentage of oviposition by *Plutella xylostella* on broccoli leaves treated with commercially available plant extracts. Bars with the asterisk are not statistically different from the control (chi-square method).

For residual activity trials, only three plant extracts (Bio Crack, Garlimax, and Nimicide 80) and the absolute control (Inex A + distilled water) were evaluated. These were selected on the basis of their effectiveness in toxicity, repellency, and oviposition inhibition trials.

Residual activity in the inhibition of *P. xylostella* feeding. In the residual feeding inhibition trial, significant differences among treatments were observed at 2 d after application (DAA) ($F_{3,28} = 4.25$, $P = 0.013$). Leaves treated with Garlimax recorded the lowest percentage of consumed area (15.8%), followed by Bio Crack and Nimicide 80, with 16.6% and 20.1% consumption, respectively, whereas in the absolute control, larvae consumed 23.5% of the leaves. At 4 DAA, no significant differences were noted among treatments ($F_{3,28} = 1.58$, $P = 0.215$). However, a similar trend to the previous assessment was observed, as Garlimax caused the lowest percentage of leaf area consumed (14.9%), whereas Bio Crack and Nimicide 80 obtained 16.2% and 15.7%, respectively. At 6, 8, and 10 DAA, no significant differences were obtained in the consumed area of treated leaves compared with the control (6 d: $F_{3,28} = 0.07$, $P = 0.974$; 8 d: $F_{3,28} = 0.740$, $P = 0.537$; 10 d: $F_{3,28} = 4.81$, $P = 0.964$) (Fig. 3).

Residual activity in the inhibition of *P. xylostella* oviposition. In the residual oviposition inhibition trial, significant differences among treatments were observed at 2 DAA ($F_{3,36} = 6.99$, $P < 0.0001$). Leaves treated with the absolute control recorded the highest number of eggs (18.3), whereas Garlimax reported the lowest number of eggs oviposited (6.6). Other plant extracts that registered fewer ovipositions were Bio Crack and Nimicide 80, with 10.2 and 9.6 eggs, respectively. At 4 DAA, no significant differences were found in oviposition inhibition among the

Table 2. Visitation rate of of *Plutella xylostella* adults on broccoli leaves treated with commercial plant extracts.

	Treatment	Visitation Rate (%)				
		Treated Leaf	Untreated Leaf	χ^2	df	*P* value
T1	Bio Crack	52.34	47.66	133.04	9	<0.0001
T2	Garlimax	12.59	87.41	220.61	9	<0.0001
T3	Bio Capsi	42.29	57.71	239.46	9	<0.0001
T4	Biodi E	27.58	72.42	110.03	9	<0.0001
T5	Nimicide 80	16.03	83.97	220.19	9	<0.0001
T6	Biotika Ricinus	37.72	62.28	147.64	9	<0.0001
T7	Organoil Plus	45.12	54.88	190.1	9	<0.0001
T8	CinnAcar	28.19	71.81	201.8	9	<0.0001
T9	Natural King	28.65	71.35	99.87	9	<0.0001
T10	Exalt	46.13	53.87	31.55	9	<0.0001

treatments ($F_{3,36} = 2.48$, $P = 0.0762$). Garlimax and Nimicide 80 recorded the lowest number of eggs laid, with 6.7 and 6.9, respectively, followed by Bio Crack, which had 9.0 eggs; the absolute control recorded the highest number of eggs (13.0). In evaluations conducted at 6, 8, and 10 DAA, no significant differences were observed among treatments (6 d: $F_{3,36} = 1.13$, $P = 0.349$; 8 d: $F_{3,36} = 1.24$, $P = 0.308$; 10 d: $F_{3,36} = 1.22$, $P = 0.315$) (Fig. 4).

Discussion

Among the nine treatments evaluated, the neem extract Nimicide 80 exhibited the highest toxicity against third-instar larvae of *P. xylostella* at 72 h (35%), which aligns with Liang et al. (2003), who reported 40% mortality with neem extracts (Agroneem, Ecozin, and Neemix). The toxicity of neem may be attributed to its tetranortriterpenoid compound, which blocks the cell cycle at the mitotic cell level and induces apoptosis (Huang et al. 2011, Salehzadeh et al. 2003). Additionally, neem can induce thermal shock in genes and proteins, altering their function (Ahmad 2012, Mordue et al. 2005). Neem has been reported to have a deterrent effect on oviposition, as a repellent, antifeeding, growth retardant, and molt inhibitor (Mordue and Blackwell 1993; Schmutterer 1990, 1995). The toxicity of neem also has been reported in lepidopterans such as *Spodoptera exigua* (Hübner), besides *P. xylostella* (Liang et al. 2003, Sharma and Singh 2014, Viana and Prates 2003).

Regarding oviposition inhibition, among the evaluated commercial plant extracts, the garlic extract Garlimax inhibited oviposition by 80%, which is likely associated with a repellent effect. This repellent effect on *P. xylostella* may be attributed to its action on the olfactory receptor neurons of insects, playing a crucial role in their

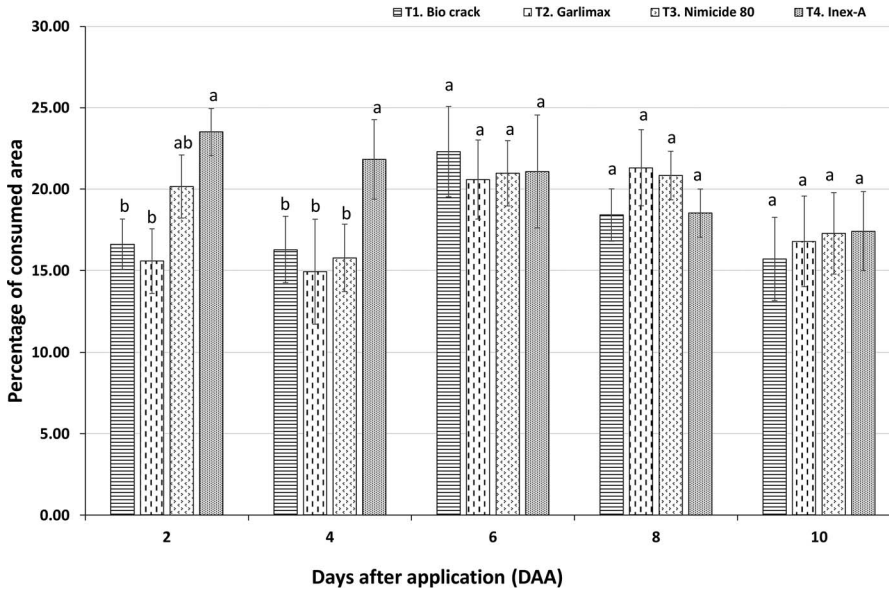

Fig. 3. Residual effect on feeding inhibition in *Plutella xylostella* with commercial plant extracts. Bars with the same letters are not statistically significant ($P \leq 0.05$).

chemical response (Masse et al. 2009). Insects heavily rely on their chemical senses to locate vital resources such as mates and oviposition or feeding sites (de Bruyne and Baker 2008). Olfactory receptors in female antennae lead the insect to avoid potentially hazardous places for their offspring (Formisano et al. 2013). Inhibition of oviposition and repellency for feeding with garlic extract has been reported in species such as *Oligonychus coffeae* (Nietner) (Acari: Tetranychidae) and *Musca domestica* (L.) (Diptera: Muscidae), as well as in *P. xylostella* (Roobakkumar et al. 2010, Samarasinghe et al. 2007).

For the residual assays, the results indicate consistency in effects at 2 and 4 DAA and an absence of effects from plant extracts and conventional insecticide at 6, 8, and 10 DAA, as statistically significant differences were not detected. The plant extracts inhibited oviposition and feeding up to 4 DAA, falling within the range observed by Schmutterer (1990) and Kodjo et al. (2011), who indicated that neem and ricinus extract residues can persist for 4 to 8 d, whereas extracts based on *Annona squamosa* L. (Annonaceae) showed residual activity of 2 to 3 d with *P. xylostella* control (Leatemia and Isman 2004). In other studies where *Pachyrhizus erosus* L. (Fabaceae) seed extract was evaluated, a residual repellent or dissuasive effect on *Plutella xylostella* was observed at 3 d, which is associated with the volatile nature of the extract (Basukriadi and Wilkins 2014). Garlic-based extracts on *P. xylostella* have demonstrated little persistence because of the rapid degradation of the active ingredient, allicin (Koch and Lawson 1996).

In conclusion, the neem extract Nimicide 80 showed toxicity against third-instar larvae of *P. xylostella*, causing a mortality rate of 35%, whereas the product Garlimax

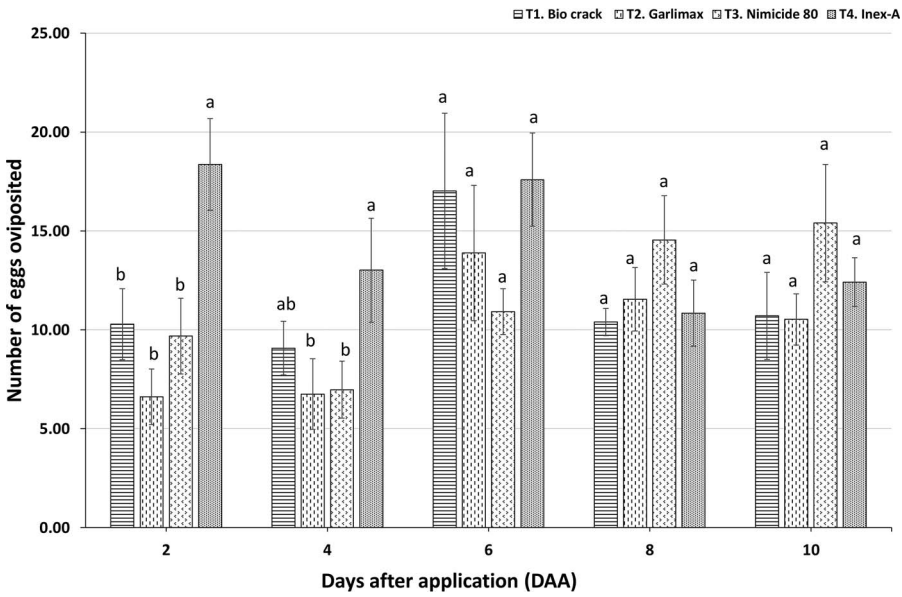

Fig. 4. Residual effect on oviposition inhibition of *Plutella xylostella* on broccoli leaves treated with commercially available plant extracts. Bars with the same letters are not statistically significant ($P \leq 0.05$).

was the extract that inhibited oviposition by 80% and achieved 85% repellency. The remaining commercial plant extracts and the conventional insecticide caused repellency of less than 71%. In the residuality assays and their effects on oviposition and feeding inhibition, Garlimax recorded the lowest number of eggs laid and less leaf area consumption at 2 and 4 DAA. Although the results are promising, field studies are recommended to determine the real potential of these products in managing *P. xylostella*.

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