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Insecticidal activity of indole derivatives against *Plutella xylostella* and selectivity to four non-target organisms

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Abstract

The diamondback moth *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) is a destructive pest of brassica crops of economic importance that have resistance to a range of insecticides. Indole derivates can exert diverse biological activities, and different effects may be obtained from small differences in their molecular structures. Indole is the parent substance of a large number of synthetic and natural compounds, such as plant and animal hormones. In the present study, we evaluate the insecticidal activity of 20 new synthesized indole derivatives against *P. xylostella*, and the selectivity of these derivatives against non-target hymenopteran beneficial arthropods: the pollinator *Apis mellifera* (Linnaeus, 1758) (Hymenoptera: Apidae), and the predators *Polybia scutellaris* (White, 1841), *Polybia sericea* (Olivier, 1791) and *Polybia rejecta* (Fabricius, 1798) (Hymenoptera: Vespidae). Bioassays were performed in the laboratory to determine the lethal and sublethal effects of the compounds on *P. xylostella* and to examine their selectivity to non-target organisms by topical application and foliar contact. The treatments consisted of two synthesized derivatives (most and least toxic), the positive control (deltamethrin) and the negative control (solvent). The synthesized compound **4e** [1-(1*H*-indol-3-yl)hexan-1-one] showed high toxicity (via topical application and ingestion) and decreased the leaf consumption by *P. xylostella*, displaying a higher efficiency than the pyrethroid deltamethrin, widely used to control this pest. In addition, the synthesized indole derivatives were selective to the pollinator *A. mellifera* and the predators *P. scutellaris*, *P. sericea* and *P. rejecta*, none of which were affected by deltamethrin. Our results highlight the promising potential of the synthesized indole derivatives for the generation of new chemical compounds for *P. xylostella* management.

Keywords Chemical control · Pest control · Pesticides · Tryptamine

Introduction

Plants of the Brassicaceae family (e.g., *Brassica oleracea*: cabbage, cauliflower, Brussel sprouts, headed cabbage and broccoli) are important food sources (Higdon et al. 2007) due to their nutritional properties and anticarcinogenic activities (Hasler 1998; Souza et al. 2003). While such attributes have motivated the large-scale production of brassicas (Novo et al. 2010), the production costs are high because these plants are attacked by agricultural pests, among which, the diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae), a brassica specialist insect, stands out. The global economic losses resulting from the attack of this pest are estimated at around US$4–5 billion per year (Zalucki et al. 2012; Correa-Cuadros et al. 2014). Moreover, the inappropriate use of insecticides to minimize these losses has caused resistance in *P. xylostella* to a range of chemical compounds (Bortoli et al. 2013).

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Populations of *P. xylostella* have shown resistance to almost all classes of insecticides applied in the field (Bortoli et al. 2013; Lin et al. 2017), including the pyrethroids widely used for its control, as well as the recently synthesized products (e.g., chlorantraniliprole and flubendiamide) (Troczka et al. 2012). In addition, pyrethroids have demonstrated high toxicity to non-target organisms, such as the agents of biological control (predatory wasps [Galvan et al. 2002; Fernandes et al. 2008; Bacci et al. 2009]) or pollinators (bees [Sharma and Abrol 2005; Fernandes et al. 2008]). In order to minimize this problem, several studies have searched for alternative control products that are relatively more efficient and environmentally safer. Thus, synthesized compounds have been highlighted as promising tools in IPM (Integrated Pest Management) (Alvarenga et al. 2012; Feng et al. 2012; Lima et al. 2015; Sun and Zhou 2015; Zhang et al. 2015; Yang et al. 2016).

The synthesis of compounds allows creating new molecules with similar structures to each other, but that can have different insecticidal activity efficiency. The indole is a parent substance (e.g., indole and tryptamine) of several derivates. Indole-based compounds are common in natural products (e.g., essential amino acids, plant and animal hormones, bacteria metabolism) and in small synthetic molecules, presenting a wide range of biological activities (Kaushik et al. 2013, Song et al. 2016) similar to those observed in plant defense mechanisms (Ahuja et al. 2015) against insects. Indole derivatives seem to act in the kynurenine pathway, which presents intermediate compounds with paralytic and lethal action on insects (Cerstiaens et al. 2003). In this pathway, the strong neurotoxic action is associated with the accumulation of 3-hydroxy-kynurenine (3-HK) in the organism, causing apoptosis of neuronal cells and motor disorders in insects (Okuda et al. 2002; Cerstiaens et al. 2003). The levels of 3-HK in the insect's physiology is regulated by a transaminase reaction catalyzed by transaminase 3-HK, which converts it to the stable xanthurenic acid (Rossi et al. 2006; Han et al. 2007). Therefore, toxic levels of 3-HK could be achieved by 3-HK transaminase inhibitors (Rossi et al. 2006), a role that can be played by the indole derivatives.

In the present study, we evaluate the insecticidal activity of new indole derivatives synthesized from indole and tryptamine against larvae of *P. xylostella*; as well as their selectivity to beneficial arthropods (e.g., predator wasps and pollinator honey bee).

Material and methods

Synthesis of the indole derivatives

All indole derivatives were synthesized at the Laboratory of Medicinal Chemistry of the Federal University of Sergipe (UFS), São Cristóvão, SE, Brazil. Derivatives **2a** to **2i** were synthesized according to the procedure of Roszkowski et al. (2005), wherein tryptamine is reacted with acid chloride derivatives in the presence of dichloromethane (DCM) and triethylamine to give substituted 1*H*-indol-3-ethylamine (Scheme 1a).

The acylated derivatives **4a** to **4j** were synthesized from indole via the regioselective Friedel–Crafts reaction at C3 of the pyrrole ring in the presence of acyl chloride, DCM and tin chloride ($SnCl_4$) as the catalyst (Ottoni et al. 2001) (Scheme 1b). Derivative **5** was obtained from the reaction of indole in the presence of tosyl chloride, NaOH and benzyltriethylammonium chloride in DCM. The reactions were prepared under anhydrous conditions and monitored by thin layer chromatography, in which the starting material was analyzed for comparison. Additionally, the reactions were processed by extraction, drying with sodium sulfate (Na_2SO_4) and solvent evaporation under reduced pressure. The obtained compounds were purified by column chromatography with silica gel as the stationary phase and hexane:ethyl acetate (8:2) as the mobile phase. Derivatives with linear, branched aliphatic chains containing chlorine atoms and a substituted aromatic derivative were synthesized. Nine compounds derived from tryptamine, and 11 derived from indole, were obtained, totaling 22 compounds, along with their base substances (tryptamine and indole).

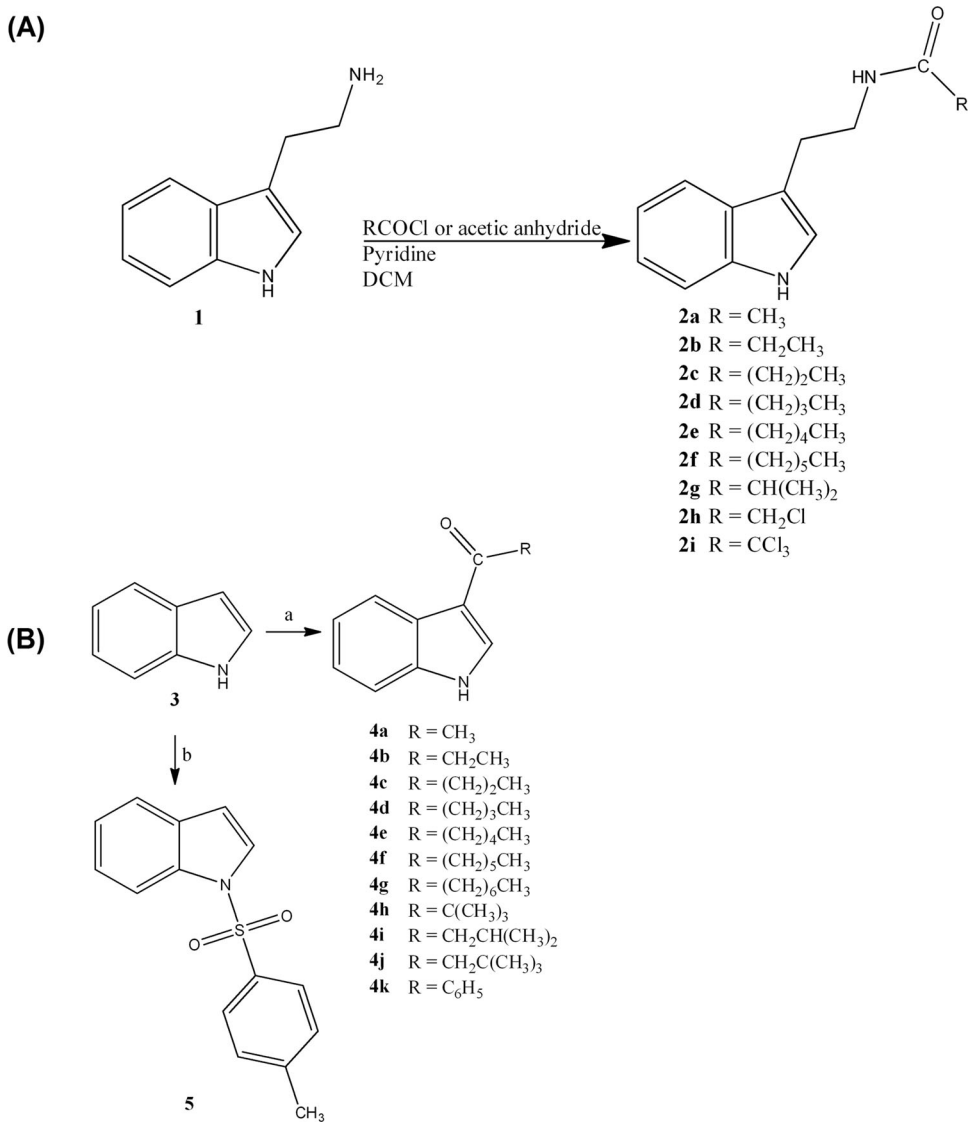
Preparation of solutions

All synthesized compounds and the insecticide were previously weighed in a precision balance with 0.01 mg sensitivity (AUW220D, Shimadzu) and were then diluted in Tween 80 surfactant (Dinâmica®, Diadema, SP, Brazil) (4% v/v), followed by homogenization. Subsequently, dimethylsulfoxide (DMSO) (Neon, Suzano, SP, Brazil) (29% v/v) was added, and a further homogenization was performed. Finally, distilled water (67% v/v) was added, and the solution was again homogenized. Preliminary tests were performed and demonstrated that the solvent components (Tween + DMSO + distilled water) did not affect the survival and behavior of insects in the proportions used.

Bioassays

Bioassays were carried out at the Agricultural Entomology Laboratory of UFS (10°54′S, 37°04′W). The experimental designs were completely randomized.

Initially, the lethal doses (LDs) of 20 synthesized compounds were evaluated for *P. xylostella*. Among these compounds, the most and least toxic were selected for analysis in the subsequent bioassays since they represented the range of activity of all the compounds analyzed. All bioassays consisted of four treatments: two synthesized



Scheme 1 **a** Preparation of tryptamine amide derivatives, and **b** preparation of indole derivatives

derivatives (most and least toxic), a positive control (commercial product based on deltamethrin (Decis® 25 EC, Bayer, Gujarat, India) and a negative control (solvent).

Bioassays with Plutella xylostella

Obtaining the larvae Larvae of *P. xylostella* were obtained from rearing, established in the Laboratory of Agricultural Entomology of UFS. The colony started with eggs, larvae and pupae collected in cabbage crops (*B. oleracea* var. capitata), in the region of Itabaiana, SE, Brazil (10°47′S, 37°22′W, and altitude of 215 m). The eggs and larvae were kept in plastic containers (2L) covered with organza mesh under ambient conditions. Cabbage leaves were offered to larvae. The plants were grown under greenhouse conditions, without application of agrochemicals. Honey and cabbage leaves were available for adult feeding and oviposition of females, respectively. The leaves containing the eggs were used to start a new cycle.

For the bioassays, 2nd instar larvae were used, based on the size and color of the cephalic capsule. The only exception was made in the avoidance behavior bioassay, in which 3rd instar larvae were used.

Lethal dose (LD) For calculations of the dosages to be used in the bioassays, initially, the average mass of 50 *P. xylostella* larvae was obtained using a precision analytical balance (AUW220D, Shimadzu). Preliminary tests were performed using three doses of all the synthesized indole derivatives and the insecticide (0.1, 1.0 and 2.5 µg of substance/mg of insect). From these tests, at least six doses were determined from the dose–mortality curves. Four replicates were performed for each treatment ($N = 96$).

Bioassays were conducted in Petri dishes (Global Trade Technology, Monte Alto, SP, Brazil) (6.0 cm diam. × 1.5 cm ht.) covered with moistened filter paper (UniFil, Alvorada, RS, Brazil) (0.3 mL of distilled water) and containing a cabbage leaf disc (2.5 cm). Ten larvae, treated with 0.5 µL of solution using a microsyringe (Hamilton®, Reno, NV, USA), were placed on each leaf disk. The Petri dishes containing treated larvae were covered with plastic film (Dispafilm, Guarulhos, SP, Brazil) and kept in a biochemical oxygen demand (B.O.D.) incubator (Biotech®, Piracicaba, SP, Brazil) at 25 ± 1 °C, >70 ± 10% relative humidity and 12-h photoperiod (12-h L:12-h D). Evaluations of mortality were performed 24 h after beginning the bioassays. The insects were considered dead when they remained immobile after being touched with a fine paintbrush.

Survival analysis The LD_{90} obtained for the treatments in the LD bioassay were used to determine the survival curves and lethal times (LTs) required to kill 50% of the population (LT_{50}). The procedures were the same as those adopted in the bioassays of LD; however, 10 repetitions were performed for each treatment ($N = 40$). The mortality of individuals was evaluated every 2 h in the first 24 h, followed by 4-h intervals in the following 24 h, and, after that, every 12 h until 20% larval mortality was achieved in the negative control.

Lethal concentration Preliminary tests were performed using three concentrations (0.1, 1.0 and 10%) of the treatments. Subsequently, six concentrations (between the minimum and maximum mortality interval) were determined to obtain the concentration–mortality curves. Four replicates were performed for each treatment ($N = 96$).

Bioassays were conducted in Petri dishes, as described in the LD bioassays. The leaf discs of cabbage were dipped in solutions for 10 s. After 30 min, the dried discs were transferred to Petri dishes. The evaluations of mortality were performed 48 h after beginning the bioassays.

Feeding behavior Leaf cabbage consumption was determined following the same procedures used in lethal concentration (LC) bioassays, using the lower sublethal concentration of the treatment's solution (0.1%). After 48 h, the leaf discs were collected and photographed. The leaf area consumed was quantified using NIS Elements software (version 4.5).

Avoidance behavior Choice bioassays were performed in Petri dishes (6.0 cm diam. × 1.5 cm ht.) lined with moist filter paper (0.3 mL of distilled water) and containing a cabbage leaf disc (6 cm). Each leaf disc was made so that the leaf's central vein delimited two areas of equal size. Half of the leaf disc was treated with the negative control, and the other with a solution at the LC_{50} of the treatments. Each half of the disk was carefully immersed in the solution for 10 s, and after 30 min, were transferred dry to the Petri dish. Sixty repetitions were performed for each treatment, totaling 240 arenas.

In the center of each arena, a larva previously marked with red, non-toxic textile ink (Acrilex Special Tapes S.A., São Bernardo do Campo, SP, Brazil) was released 1 h before starting the bioassay. In each arena, the walking of larva was recorded for 30 min using a video camera (Panasonic SD5 Super Dynamic WV-CP504) equipped with a Spacecom lens (1/3″ 3–8 mm) coupled to a computer with EthoVision XT software (version 8.5; Noldus Integration System, Sterling, VA, USA). Data were analyzed in the Studio 9 software (Pinnacle Systems, Mountain View, CA, USA). The duration of insects in the treated and non-treated areas was recorded.

Bioassays with non-target hymenopteran species

Obtaining insects Active nests of *P. scutellaris*, *P. sericea* and *P. rejecta* were collected at the *campus* of UFS. *A. mellifera* foraging bees were collected in the municipality of Barra dos Coqueiros, SE, Brazil (10°48′S, 36°58′W, and altitude of 14 m). The wasp nests and the honeybees were kept, separately, in plastic containers (7L), covered with organza mesh under ambient condition. The honeybees were fed with 50% sucrose solution. No food was provided to wasps.

Bioassays were performed in Petri dishes (9.0 cm diam. × 1.5 cm ht.) covered by filter paper and containing one glass vial (1.5 × 0.7 cm) with 0.5 mL of 50% sucrose solution. Each bioassay repetition consisted of seven treated individuals for each Petri dish. Insects were considered dead when they remained immobile after being touched with a fine paintbrush.

Selectivity by topical application In the pronotun region of each insect, a 1 µL aliquot of one of the treatments was applied, using a microsyringe. For all treatments, the solutions were applied at the predetermined LD_{90} for *P. xylostella*. The treated individuals were previously kept in a freezer at −10 °C for 2 min in order to reduce their activity. The Petri dishes containing the treated insects were covered by a plastic film with small holes for ventilation. Evaluations of mortality were performed 24 h after beginning the bioassays.

Selectivity by foliar contact Cabbage leaf discs were immersed for 10 s in solutions of treatments at the predetermined LC_{90} for *P. xylostella*. After 30 min, the completely dry leaf discs were transferred to Petri dishes,

followed by the individual insects. Evaluations of mortality were performed 48 h after beginning the bioassays.

Statistical analysis

Data of mortality were submitted to probit analyses to determine the dose–mortality and concentration–mortality curves of each compound, using the PROC PROBIT procedure. From these curves, the LDs and LCs required to cause 50% (LD_{50}) and 90% (LD_{90}) of mortality, and their respective confidence intervals at 95% of probability (CI_{95}), were obtained. The LDs and LCs were compared by the criterion of not overlapping the CI with the origin of the interval.

The survival analyzes were performed using Kaplan–Meier estimates from the proportion of surviving insects at the beginning to the end of the experiment, using the log-rank test. From these analyzes, survival curves and LTs to cause mortality in 50% of individuals (LT_{50}) were obtained. Differences among these curves were verified by the Holm–Sidak multiple comparison method ($P < 0.05$) (SigmaPlot 11.0).

The response variables, namely, leaf consumption, avoidance behavior of *P. xylostella* larvae and the selectivity of non-target Hymenoptera, were analyzed using analysis of variance (ANOVA), followed by Tukey's test ($P < 0.05$) (PROC GLM with Tukey's test; SAS). Data were previously analyzed for conformity with the assumptions of normality, using SAS software (SAS 2008).

Results

Bioassays with Plutella xylostella

Lethal dose

All indole derivatives were toxic, by contact, to *P. xylostella* larvae. Derivative **2a**, obtained from tryptamine, exhibited the lowest toxicity among all tested compounds ($LD_{50} = 6.33\ \mu g\ mg^{-1}$). Instead, compound **4e**, synthesized from indole, exhibited the highest toxicity among all compounds ($LD_{50} = 0.42\ \mu g\ mg^{-1}$) (Table 1).

Among the compounds obtained from tryptamine, six (**2b**, **2d**, **2e**, **2f**, **2h** and **2i**) were more toxic than tryptamine. The other compounds (**2a**, **2c** and **2g**) showed toxicity similar to tryptamine. The highest activity was produced by compound **2e** ($LD_{50} = 0.70\ \mu g\ mg^{-1}$), which was 7.97-fold more active than tryptamine (Table 1).

Eight compounds (**4b**, **4c**, **4e**, **4g**, **4h**, **4i**, **4j** and **5**) synthesized from indole were more active than indole, with emphasis on compound **4e** ($LD_{50} = 0.42\ \mu g\ mg^{-1}$), which displayed 12.50-fold higher activity. Compounds **4a**, **4d** and **4f** presented similar potencies to indole (Table 1).

Compound **4e** showed higher insecticidal activity than deltamethrin while three compounds (**2d**, **2e** and **2i**) showed similar potency to deltamethrin. However, the remaining evaluated derivatives were less active than deltamethrin (Table 1).

Survival analysis

The survival of *P. xylostella* larvae, exposed to the most (**2a**) and least toxic (**4e**) compounds at the LD_{90} was significantly reduced over time (log-rank test: $\chi^2 = 205.69$; *d.f.* $= 2$; $P < 0.001$) (Fig. 1).

Deltamethrin exhibited the lowest lethal time, followed by compounds **4e** and **2a**. The survival curves of compounds **2a** and **4e** differed from each other, whereas, there was no significant difference between the curves of deltamethrin and the compound **4e** ($P < 0.05$) (Fig. 1).

Lethal concentration

Derivatives **2a** and **4e** were toxic to the *P. xylostella* larvae. The LC_{50} of both substances showed significantly higher activity than deltamethrin. The compound **4e** ($LC_{50} = 0.017\ mg\ mL^{-1}$) was around 8- and 20-fold more potent than **2a** ($LC_{50} = 0.14\ mg\ mL^{-1}$) and deltamethrin ($LC_{50} = 0.33\ mg\ mL^{-1}$), respectively.

Analysis of the LC_{90} values revealed that compound **4e** ($LC_{90} = 0.08\ mg\ mL^{-1}$) showed higher potency, followed by deltamethrin ($LC_{90} = 0.44\ mg\ mL^{-1}$) and **2a** ($LC_{90} = 0.84\ mg\ mL^{-1}$) (Fig. 2).

Feeding behavior

The leaf consumption by *P. xylostella* larvae was significantly different among treatments ($F_{3,12} = 48.16$; $P < 0.03$). The lowest consumption was observed when the plant was treated with compound **4e** ($154.11 \pm 13.44\ mm^2$), followed by deltamethrin ($232.75 \pm 17.91\ mm^2$) and **2a** ($309.31 \pm 34.97\ mm^2$) when compared with the control ($490.87 \pm 0.00\ mm^2$) (Fig. 3).

Avoidance behavior

Compounds **2a** and **4e** did not induce avoidance behavior in *P. xylostella* larvae, and there was no significant difference in the time spent by individuals between treated and non-treated areas (**2a**: $F_{1,118} = 0.06$, $P = 0.80$; **4e**: $F_{1,118} = 0.99$, $P = 0.32$). However, larvae avoided the areas treated with deltamethrin ($F_{1,118} = 21.35$; $P < 0.02$), spending 70% of the time in the non-treated area (Fig. 4).

Table 1 Toxicity by topical application of the indole derivatives on *Plutella xylostella* after 24 h of exposure

Code	Compounds	No of insects	LD_{50} ($CI_{95\%}$) (μg mg^{-1})	LD_{90} ($CI_{95\%}$) (μg mg^{-1})	Slope	χ^2	*P*
1	Tryptamine	280	5.58 (4.42–6.84)	43.46 (25.99–117.17)	1.44	0.50	0.78
2a	*N*-(2-(1*H*-indol-3-yl)ethyl) acetamide	240	6.33 (5.67–7.13)	18.60 (14.67–26.84)	2.74	0.45	0.80
2b	*N*-(2-(1*H*-indol-3-yl)ethyl) propanamide	320	2.26 (1.86–2.74)	13.10 (9.51–20.20)	1.68	1.39	0.50
2c	*N*-(2-(1*H*-indol-3-yl)ethyl) butanamide	280	5.12 (3.67–7.19)	111.67 (57.47–311.84)	0.96	0.59	0.75
2d	*N*-(2-(1*H*-indol-3-yl)ethyl) pentanamide	240	0.84 (0.65–1.07)	8.59 (5.49–16.70)	1.27	1.43	0.50
2e	*N*-(2-(1*H*-indol-3-yl)ethyl) hexanamide	240	0.70 (0.44–1.04)	37.18 (15.38–171.97)	0.74	1.85	0.60
2f	*N*-(2-(1*H*-indol-3-yl)ethyl) heptanamide	240	2.46 (1.82–3.35)	39.59 (22.87–86.45)	1.06	1.49	0.52
2g	*N*-(2-(1*H*-indol-3-yl)ethyl) 2-methylpropanamide	240	4.39 (3.30–5.76)	62.68 (32.53–208.68)	1.11	2.13	0.34
2h	2-chloro-*N*-(2-(1*H*-indol-3-yl)ethyl) acetamide	240	2.50 (1.95–3.31)	23.94 (14.12–53.64)	1.30	0.09	0.95
2i	2,2,2-trichloro-*N*-(2-(1*H*-indol-3-yl)ethyl) acetamide	280	0.85 (0.65–1.10)	13.75 (8.29–28.65)	1.06	2.88	0.58
3	Indole	240	5.25 (4.70–5.90)	15.21 (12.10–21.54)	2.77	0.80	0.67
4a	1-(1*H*-indol-3-yl)ethanone	320	3.82 (3.02–4.91)	60.29 (35.52–129.50)	1.07	3.49	0.52
4b	1-(1*H*-indol-3-yl)propan-1-one	240	1.94 (1.38–2.68)	38.97 (22.97–80.16)	0.98	0.20	0.90
4c	1-(1*H*-indol-3-yl)butan-1-one	240	1.00 (0.68–1.47)	23.55 (13.28–49.23)	0.93	0.77	0.68
4d	1-(1*H*-indol-3-yl)pentan-1-one	240	4.53 (4.18–4.93)	9.51 (8.24–11.59)	3.97	0.23	0.89
4e	1-(1*H*-indol-3-yl)hexan-1-one	240	0.42 (0.32–0.50)	2.41 (1.61–5.29)	1.68	2.07	0.36
4f	1-(1*H*-indol-3-yl)heptan-1-one	280	5.00 (4.57–5.52)	12.73 (10.78–15.83)	3.16	1.73	0.63
4g	1-(1*H*-indol-3-yl)-2,2-dimethylpropan-1-one	320	1.27 (0.95–1.65)	24.10 (15.69–43.23)	1.00	3.30	0.51
4h	1-(1*H*-indol-3-yl)-3-methylbutan-1-one	240	3.62 (3.00–4.48)	18.92 (13.17–31.55)	1.78	0.59	0.75
4i	1-(1*H*-Indol-3-yl)-3,3-dimethyl-butan-1-one	280	2.37 (1.88–3.01)	21.63 (13.81–41.80)	1.33	1.51	0.53
4j	1-(1*H*-indol-3-yl)(phenyl)methanone	240	2.77 (2.23–3.32)	16.20 (11.61–27.26)	1.67	1.64	0.55
5	Tosyl indole	280	2.50 (2.27–2.76)	7.05 (5.84–9.22)	2.85	2.28	0.52
	Deltamethrin	240	1.19 (0.90–1.56)	11.28 (7.67–18.78)	1.31	1.16	0.56

LD_{50} = lethal dose required to kill 50% of population

LD_{90} = lethal dose required to kill 90% of population

$CI_{95\%}$ = Confidence intervals at 95%

Bioassays with non-target hymenopteran species

Selectivity by topical application

The survival of *A. mellifera*, *P. scutellaris*, *P. sericea* and *P. rejecta* did not differ from the control treatment when topically treated with the LD_{90} of compounds **2a** and **4e** for *P. xylostella*. The percentage of surviving individuals exposed to both derivatives was higher than 85% for all species, differing significantly from deltamethrin, which showed only 5% survival of individuals (Fig. 5a).

Selectivity by foliar contact

The survival of individuals of *A. mellifera*, *P. scutellaris*, *P. sericea* and *P. rejecta* did not differ from the control after leaf contact with the LC_{90} of compounds **2a** and **4e**. For all species tested, the percentage of surviving insects exposed to the two synthesized substances was higher than 85%, differing significantly from deltamethrin treatment, in which there were no survivors (Fig. 5b).

Discussion

The present study highlights the efficacy of indole derivatives for the control of *P. xylostella*, emphasizing compound **4e** [1-(1*H*-indol-3-yl)hexan-1-one], which was more efficient than deltamethrin, a product that has been causing induction of resistance in several populations of this insect pest. The compound **4e** showed high selectivity to target species rather than different non-target hymenopteran species when compared with the synthetic insecticide deltamethrin.

Synthesized indole derivatives showed insecticidal activity against *P. xylostella* larvae. Studies have revealed that biological activity may be modulated by the presence of certain chemical chains or groups present in the structure of a compound (Santos et al. 2011; Barbosa et al. 2012; Oliveira et al. 2014; Zhou et al. 2014; Lima et al. 2015; Nguyen et al. 2015; Gallardo et al. 2016). Some research also predicts a greater biological activity by molecular structures with shorter chains than longer chains (Nguyen et al. 2015; Gallardo et al. 2016). In the present study, all

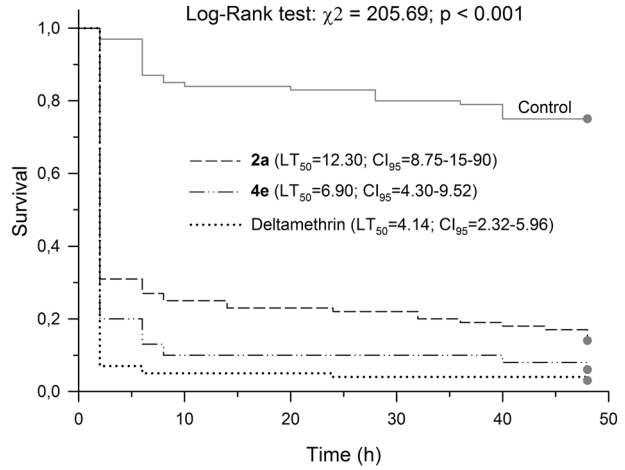


Fig. 1 Survival curves and mean lethal time (LT_{50}) of *Plutella xylostella* exposed via topical application to the lethal dose (LD_{90}) of the more and less toxic synthesized derivatives (**4e** and **2a**, respectively). LT_{50} = lethal time required to kill 50% of the population

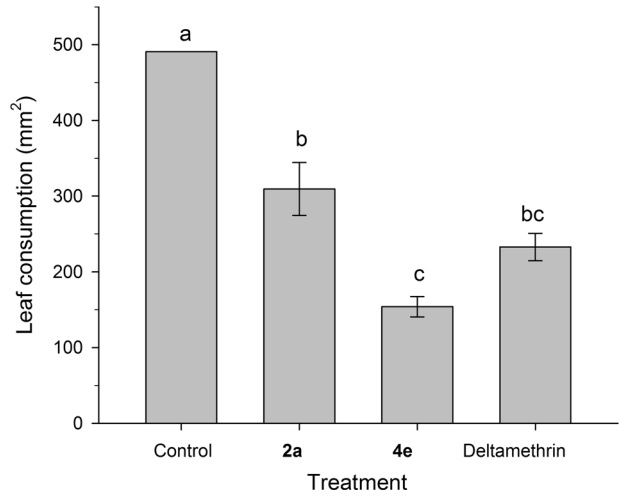


Fig. 3 Leaf consumption by *Plutella xylostella* exposed via ingestion to the solutions (0.1%) of the more and less toxic derivatives (**4e** and **2a**, respectively) after 48 h. Means followed by the same letters do not differ by Tukey's test ($P < 0.05$)

synthesized compounds exhibited insecticidal activity, either higher or similar to their respective parent materials (indole and tryptamine), but their activities were independent of the length of the chains. These variations in the biological activity of members of a homologous series could be related to the addition of different chemical groups that interfere with at least one of the following processes: (i) specific drug–receptor interaction related to target sites; (ii) insect metabolism through excretion of bioactive substances (Ing 1964); and (iii) changes in physical properties, such as solubility (Raison and Standen 1955).

The susceptibility of the *P. xylostella* larvae to **4e** was superior to the other treatments (Table 1). This higher activity seems to be associated with its mode of action, which is

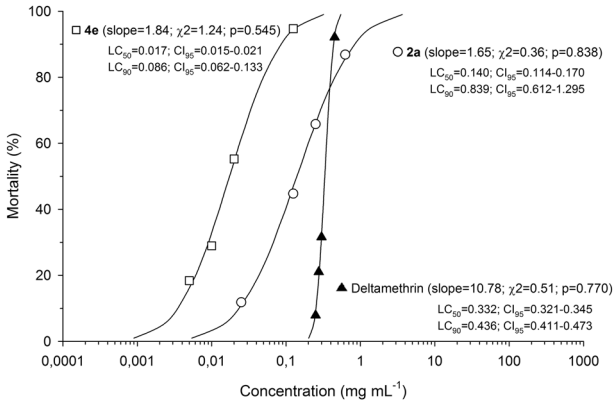


Fig. 2 Concentration-mortality curves of *Plutella xylostella* exposed via ingestion to the more and less toxic derivatives (**4e** and **2a**, respectively), after 48 h. LC_{50} = lethal concentration required to kill 50% of the population

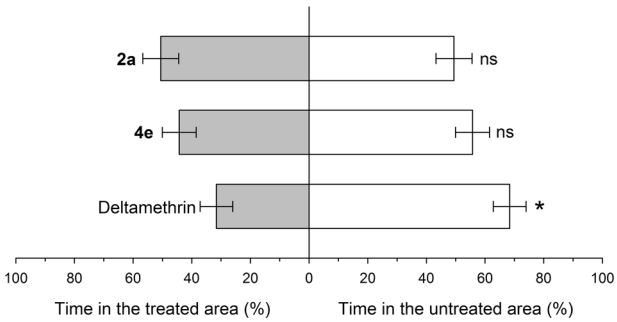


Fig. 4 Avoidance behavior of *Plutella xylostella* individuals in the function of exposure to the more and less toxic derivatives (**4e** and **2a**, respectively) for 30 min. An asterisk indicates time spent by individuals between treated and non-treated areas differs by Tukey's test ($P < 0.05$). *n.s.* = not significant

distinct from that observed by deltamethrin. Although **4e** and deltamethrin are both neurotoxic, they act via different pathways or mode of actions. The main target of the pyrethroid deltamethrin is the sodium channels in the membranes of nerve cells (Endersby et al. 2011; Du et al. 2016) while derivative **4e** appears to act on the kynurenine pathway by blocking conversion reactions of kynurenines (neurotoxic metabolites) to more stable compounds. In insects, kynurenine can cause motor dysfunction, paralysis and instant death (Cerstiaens et al. 2003). Additionally, the differences in activity between **4e** and deltamethrin against *P. xylostella* could be related to the insect's resistance to these compounds. Under natural conditions, resistance mechanisms of *P. xylostella* to indole compounds have been described only for a restricted group of substances of the defense complex (glucosinolate–myrosinase or indole glucosinolates) produced by Brassicaceae plants (Ratzka et al. 2002). Moreover, reports of *P. xylostella* resistance to the synthesized indole derivatives have not appeared in the literature, in contrast to the plethora of research on deltamethrin.

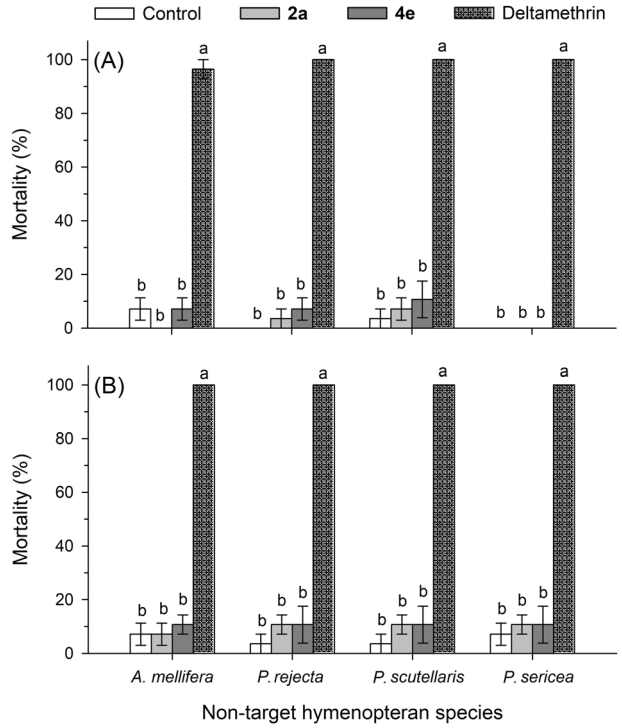


Fig. 5 Selectivity of *Polybia scutellaris*, *Polybia sericea*, *Polybia rejecta* and *Apis mellifera* exposed via topical application (**a**) and leaf contact (**b**) to the more and less toxic derivatives (**4e** and **2a**, respectively) and deltamethrin after 24 h. Means followed by the same letters do not differ by Tukey's test ($P < 0.05$)

As observed with deltamethrin, derivatives **2a** and **4e** showed rapid insecticidal action against *P. xylostella* larvae over time (Fig. 1). The higher efficiency of compound **4e** compared with **2a** may be the result of the blocking rate of the kynurenine stabilization reaction, requiring less time to produce toxicity against the insect. In addition to contact toxicity, compounds **2a** and **4e** also showed insecticidal activity by ingestion (Fig. 2), reduced the leaf consumption by the insects (Fig. 3) and did not cause escape behavior of *P. xylostella* larvae (Fig. 4). Although the food channel of lepidopteran larvae constitutes a barrier to insecticidal action, certain toxic agents can overcome this barrier by morphological alterations in the middle intestine or by the insensitivity of the detoxifying/digestive enzymes, as already observed in *P. xylostella* (Correia et al. 2009; Ramya et al. 2016). Indole derivatives promote insect neurotoxicity via the accumulation of kynurenines in the nervous system (Han et al. 2002; Rossi et al. 2006; Han et al. 2007), causing paralysis and severe or irreversible motor dysfunction (Cerstiaens et al. 2003). The reduced feeding behavior of *P. xylostella* larvae observed with compounds **2a** and **4e** seems to be associated with paralysis, caused by the accumulation of kynurenine (Chiou et al. 1998). Such toxicity, coupled with the fact that the indole compounds did not cause avoidance behavior in the larvae, suggests a possible future use for management. Individuals of *P. xylostella* were maintained on the leaves treated with compound **4e**, favoring the contact with the insecticide while reducing the leaf consumption. Conversely, we observed that larvae avoided areas treated with deltamethrin, which limits their control action through ingestion. Similar avoidance behavior has also been noticed with other pyrethroids, such as gamma-cyhalothrin (Nansen et al. 2016).

In addition to presenting high toxicity potential, compounds **2a** and **4e** were also selective to *P. scutellaris*, *P. sericea*, *P. rejecta* and *A. mellifera*, by topical application and leaf contact (Fig. 5). The levels of kynurenine are normally elevated in the egg and larval stages (Han et al. 2002), reaching the peak levels in the pupa stage (Cerstiaens et al. 2003). This compound appears to be strongly associated with the paralysis and metamorphosis of insects. In adult insects, such substances are recognized as causing motor dysfunctions. Thus, initially, a certain toxic effect was expected on non-target insects (pollinators and predators). The observed tolerance may be associated with the low penetration of the insecticide in the cuticle, due to differences in solubility of the compounds or by the increased metabolism of the insecticide and modification at the target site (Guirado et al. 2009). Instead, deltamethrin did not show selectivity for the species tested by topical application or by foliar contact. The wasps and bee mortalities were higher than 95% by topical application and reached 100% by leaf contact (Fig. 5). Previous studies have also reported high toxicity of deltamethrin (Galvan et al. 2002; Santos et al. 2003; Sharma and Abrol 2005; Bacci et al. 2009) and other pyrethroids (Sharma and Abrol 2005; Fernandes et al. 2008; Bacci et al. 2009; Barros et al. 2015) for different species of wasps (Galvan et al. 2002; Fernandes et al. 2008; Bacci et al. 2009) and bees (Sharma and Abrol 2005; Fernandes et al. 2008), including *P. scutellaris*, *P. sericea* and *A. mellifera*.

Selectivity studies have great importance to minimize the negative impact of insecticides on beneficial organisms, which are essential to ensure ecosystem integrity (Potts et al. 2010). The industry and public policy have failed in the past to determine the acceptable insecticides LDs that are non-harmful to beneficial organisms, such as honey bees (Sánchez-Bayo et al. 2016). In the present study, we verify the low toxicity of indole compounds to natural enemies and pollinator. However, we caution that additional studies need to be developed in natural populations, including the lethal and sublethal effects of the compounds in different castes under field conditions.

In summary, the synthesized indole derivatives presented high insecticidal potency against *P. xylostella* and reduced the feeding by this insect. In addition, the high selectivity demonstrated for *A. mellifera*, *P. scutellaris*, *P. sericea* and

P. rejecta suggests that the synthesized indole derivatives may have a low negative impact on pollinator insects and natural enemies under natural conditions. Further studies should focus on the development of viable formulations for practical use in the field.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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