Insecticidal Activity of Synthetic Amides on *Spodoptera frugiperda*

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The phytochemistry of the genus *Piper* (Piperaceae) has been widely studied due to the biological properties of amides from these plants. In this work, we have synthesized and evaluated the toxic effect of 11 amides against the fall armyworm *Spodoptera frugiperda* larvae. The naturally occurring piperine was also evaluated. The most active amide was *N*-[3-(3′,4′-methylenedioxyphenyl)-2-(*E*)-propenoyl]piperidine with a LD_{50} of 1.07 μg mg^{-1} larvae. This amide was also evaluated by ingestion.

Key words: Cinnamoyl Amides, Insecticide, *Spodoptera frugiperda*

Introduction

The fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), presents an ample distribution over the American continent, with occurrence from Mexico to South America (Crocomo and Parra, 1985). This species feeds of diverse grass as sorghum, wheat, pastures, barley and others. It is the most important pest of maize in Brazil and can cause reduction of up to 34% in its production, being the critical period of attack about 40 days after the plantation (Cruz and Turpin, 1982).

Usually, the control of this insect is carried out with synthetic chemical products, but the abusive use of this method can generate resistance to the insecticides, incompatibility with biological control, ambient pollution and provokes poisoning in animals and also to humans. Investigation for other methods of control includes development of substances less toxic and aggressive to the environment and more selective.

The phytochemistry of the genus *Piper* (Piperaceae) has been widely studied, due to the biological properties of amides from these plants (Scott and McKibben, 1978; Gbewonyo *et al.*, 1993; Parmar *et al*., 1997). Ewete *et al.* (2000) reported the toxicity of piperine on growth and development of *Ostrinia nubilalis* Hubner (Lepidoptera: Pyralidae). Piperine had no marked effect on reducing the maximum larval weight but caused larval mortality. A crude extract of *P. guineense* reduced the maximum larval weight at 30–300 ppm and prolonged larval and adult emergence periods.

Park *et al.* (2002) have evaluated the toxicities of two piperidine alkaloids, pipernonaline and piperoctadecalidine, isolated from *P. longum* L., against five species of arthropod pests. The most potent insecticidal activities of both alkaloids (LD_{50} = 125 and 95.5 mg l^{-1}, respectively) were against *S. litura* F. (Lepidoptera: Noctuidae).

Dyer *et al.* (2003) tested three amides, piplartine, 4′-desmethylpiplartine and cenocladamide, isolated from *P. cenocladum*, against five herbivores: *S. frugiperda*, two caterpillar species (*Eois* spp.), *Atta cephalotes*, and *Paraponera clavata*. The amides had negative effects on all insects. However, for *S. frugiperda*, for example, amide mixtures caused decreased pupal weights and survivorship and increased development times. The mixture of all three amides had the most dramatic deterrent and toxic effects in all experiments, with the effects usually surpassing expected additive responses, indicating that these compounds can act synergistically against a wide array of herbivores.

Many new synthetic amides have been prepared and evaluated. Paula *et al*. (2000) have prepared a series of piperine derivatives, which were evaluated against *Ascia monuste orseis* Latr (Lepidoptera: Pieridae) among other insect species.

In this work, we have synthesized and evaluated the toxic effect of 11 amides against the fall armyworm *Spodoptera frugiperda* larvae. The naturally occurring piperine was also evaluated.

Methods and Materials

*Synthesis of amides **1–10***

All commercially available reagents were purchased from Aldrich Chemical Co. (Milwaukee, USA). Reagents and solvents were purified when necessary according to the usual procedures described in the literature. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX-200 (200 and 50 MHz, respectively) and DRX-400 instrument (400 and 100 MHz, respectively). Mass spectra were recorded on a Shimadzu GCMS-QP5000 instrument. The IR spectra were measured on a Bomem MB-102 spectrometer (Hartmann & Braun, Quebec, Canada). Analytical thin-layer chromatography was performed on a 0.25 mm film of silica gel containing the fluorescent indicator UV_{254} supported on an aluminum sheet (Sigma-Aldrich). Flash column chromatography was performed using silica gel (Kieselgel 60, 230–400 mesh, E. Merck). Gas chromatography was performed using a Shimadzu GC-17A instrument with H_2 as carrier gas and a DB-5 column. Melting points were performed in a MQAPF-301 instrument (Microquímica, Palhoça, Brazil).

General procedure

In a 50 ml round flask, equipped with a magnetic stir bar and a condenser, the appropriate cinnamic acid and $SOCl_2$ were added under nitrogen atmosphere. The mixture was heated at 50 °C for 4 h, and then anhydrous hexane (30 ml) was added. Excess of $SOCl_2$ and the solvent were removed by distillation. The solid obtained was employed in the next step without further purification.

The cinnamoyl chloride was diluted in anhydrous dichloromethane under nitrogen atmosphere and the appropriate amine was added in order to obtain the corresponding amide. After 12 h, a saturated solution of $NaHCO_3$ (3 ml) was added and the extraction was carried out with dichloromethane (3 × 3 ml). The organic phase was washed with distilled water (2 ml) followed by brine (2 ml), and dried with anhydrous Na_2SO_4. In all cases, after evaporation of the solvent, the crude product obtained was purified by flash column chromatography using a mixture of ethyl acetate/hexane as eluent. Amides **3**–**11** were analyzed by IR, MS, ^{1}H and ^{13}C NMR spectroscopy and their spectral data were identical to those of the literature (Table I).

(±)-(2E)-3-(1,3-Benzodioxol-5-yl)-N-(sec-butyl)-acrylamide (**1**): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.56 (d, J = 15.3 Hz, 1H), 7.0 (d, J = 1.5 Hz, 1H), 6.97 (dd, J = 1.5, 8.0 Hz, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.29 (d, J = 15.3 Hz, 1H), 5.99 (s, 2H), 3.33 (ddd, J = 6.0, 6.1, 13.3 Hz, 1H), 3.20 (ddd, J = 6.1, 6.3, 13.3 Hz, 1H), 1.62 (oct, J = 6.7 Hz, 1H), [1.44 (ddq, J = 5.3, 7.4, 12.6 Hz, 2H), 1.18 (ddq, J = 5.8, 7.6, 12.6 Hz, 2H),] 0.93 (d, J = 6.7 Hz, 3H), 0.92 (t, J = 7.4 Hz, 3H). – ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.3, 17.2, 27.0, 35.0, 45.4, 101.4, 106.3, 108.5, 118.4, 123.9, 129.1, 140.9, 148.2, 149.0, 166.3. – EIMS: m/z = 261 [M^+] (18), 242 (0.9), 204 (3), 190 (63), 175 (100), 145 (63), 117 (34), 89 (68). – IR (film): ν_{max} = 3303, 2963, 1643, 1492, 1445 cm^{-1}.

(2E)-3-(1,3-Benzodioxol-5-yl)-N,N-dicyclohexyl-acrylamide (**2**): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.54 (d, J = 15.3 Hz, 1H), 7.02 (d, J = 1.6 Hz, 1H), 6.98 (dd, J = 1.6, 8.1 Hz, 1H), 6.80 (d, J = 8.0 Hz, 1H), 6.66 (d, J = 15.3 Hz, 1H), 6.00 (s, 2H), 3.58 (m, 2H), 1.82–1.18 (m, 20H). – ^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.3, 26.4, 30.3, 31.9, 56.1, 57.7, 101.4, 106.2, 108.5, 118.1, 123.6, 130.0, 141.3, 148.1, 148.7, 166.6. – EIMS: m/z = 355 [M^+] (12), 272 (10), 190 (9), 176 (20), 175 (100), 148 (19), 145 (54), 117 (27), 89 (43). – IR (film): ν_{max} = 2930, 1645, 1491, 1449 cm^{-1}.

Biological activity

Larvae of *S. frugiperda* were obtained from the Insect Bioassay Laboratory of Universidade Federal de São Carlos, Brazil, and reared on artificial diets (Kasten *et al.*, 1978; Parra, 1986). They were maintained in an incubation chamber with a photophase of 12 h, (70 ± 5)% relative humidity and a temperature of (25 ± 1) °C.

In a first experimental stage, based on the methodology described by Paula *et al.* (2000), the contact toxicity of synthetic amides **1**–**11** and commercially available piperine (Aldrich) was evaluated. Groups of 10 larvae in the second instar (5-day-old) of *S. frugiperda* were transferred to glass Petri dishes. The average weight of insects was obtained by measuring, on an analytical balance, the mass of five groups containing 10 insects each. To each individual insect 1 μl of solution of the test compound in acetone was applied topically, via a microsyringe, at concentrations of 10^{-2}, 10^{-1}, 1 and 10 mg ml^{-1} for all amides; amides **1**, **10** and piperine were also tested at the concentra-

tion of 10^2 mg ml^{-1}. Thus, the final dose was $4 \cdot 10^{-2}$, $4 \cdot 10^{-1}$, 4, 40 and $4 \cdot 10^2$ μg mg^{-1} of larvae. In order to avoid the possible insect inanition, each group of larvae was supplied with a small amount (300 mg) of artificial diet. This procedure was performed 1 h after application of the test compound. The control was carried out under the same conditions; 1 μl of acetone was applied on each insect. The mortality counts were made after 48 h. All experiments and the respective control were carried out in five replicates and the LD_{50} value was determined by analysis using Polo Software. This program uses Abbott's transformation for control mortality and calculates log dose probit lines according to the process described by Finney (1971).

In a second experimental stage, amide **10**, which caused the highest mortality in the first experiment, was tested to verify its insecticidal activity and/or activity related to the feeding of the insect. For each treatment and control, 50 first instar larvae (1-day-old) of *S. frugiperda* were used. Amide **10** was administered by incorporation into an artificial diet in which bean and wheat germ are the basic ingredients (Kasten *et al.*, 1978; Parra, 1986). In order to ensure uniformity, amide **10** (dissolved in acetone) was mixed with 1.8 g of ascorbic acid (a component of the diet) and dried, using vacuum at 40 °C, in a rotary evaporator prior to its incorporation into the diet. The mixture was incorporated to the artificial diet at the final contents of 1, 10, 50 and 100 mg kg^{-1}. The control was prepared similarly as above, but without amide **10**. The diets were placed in previously sterilized glass tubes (8.5 × 2.5 cm), into which larvae of *S. frugiperda* were introduced individually. Daily observations were made and the following parameters were evaluated: 1.) duration of larval and pupal phases; 2.) duration of the life cycle (larvae to emergence of the adult); 3.) weight of pupae and 4.) percentage of dead insects (mortality) at the end of each phase. Data were submitted to an analysis of variance (ANOVA) and the averages were compared applying the Tukey test ($P \leq 0.05$). Each tube containing one insect, independent on the developing phase, was considered as one replicate; therefore the number of the replicates was different for each treatment. For evaluation of the mortality of the larval and pupal phase and total cycle, the experimental unit was constituted by mean five tubes with one larva each, with ten replications by treatment.

Results and Discussion

The synthesis of amides **1**–**11** was performed in two steps, starting from the appropriate cinnamic acid which was converted to the corresponding acyl chloride followed by addition of the appropriate amine, with yields between 32 to 86% (Fig. 1, Table I).

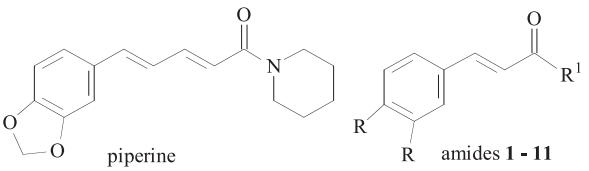

Fig. 1. Amides tested against *S. frugiperda* larvae.

The toxic effects of twelve amides on second instar larvae of *S. frugiperda* were determined. In the topical application bioassays verified that the amides **1**, **6**, **8**, **10**, **11**, and piperine caused statistically significant mean mortality relative to control, whereas the amides **2**, **3**, **4**, **5**, **7** and **9** did not present a statistic difference from the control (1%). Amides **1**, **10** and piperine caused 70, 92, and 54% of mortality, respectively, at 0.4 mg mg^{-1} larval dose. Thus, the activity of these amides varied from moderate to very satisfactory. The mortality of amides **1**, **6**, **8**, **10**, **11**, and piperine at 40 μg mg^{-1} larva dose was 56, 88, 42, 58, 54, and 42%, respectively. Amide **10** was the most promising compound as insecticide agent among the compounds studied because it was the most toxic amide to insect-pest species. The activity of amide **10** showed to be similar with the activity of piperine against *Ascia monuste orseis* (Dyer *et al*., 2003). Estrela *et al.* (2003) evaluated the toxicity of piperine analogues and also piperonyl butoxide to third instar larvae of *A. monuste orseis* and *S. frugiperda* by topical application and verified that the *N*-isopropyl amide derivative was the most active.

The data of toxicity (LD_{50}) of amides **1**, **6**, **8**, **10**, **11**, and piperine were obtained by dose-response curves (Table II); the other amides (**2**, **3**, **4**, **5**, **7** and **9**) had also been tested but the mortality did not differ from control (1%). Amides **10** and **6** provided the highest (1.07 μg mg^{-1} larvae) and the lowest (504.07 μg mg^{-1} larvae) values of LD_{50} for *S. frugiperda* larvae, respectively. The slopes of the dose-response curves indicated that the higher homogeneity of response of the pest species studied were the compounds **10**, **11** and piperine. The high slope values indicate that small variations in the

Table I. Synthetic amides **1**–**11**.

Amide	R	R^1	Yield (%)	M.p. [°C]	Lit. m. p. [°C]	Ref.
1	OCH_2CH_2O	$NHCH_2CH(CH_3)CH_2CH_3$	38	96 - 97	-	-
2	OCH_2CH_2O	$N(C_6H_{11})_2$	86	142 - 143	-	-
3	OCH_2CH_2O	$N(CH_2CH_3)_2$	42	67 - 68	70 - 71	Papa *et al*., 1950
4	OCH_2CH_2O	NHC_6H_{11}	32	181 - 182	-	Delaney *et al*., 1969
5	OCH_2CH_2O	$NHCH_2C_6H_5$	36	157 - 158	-	Rim *et al*., 1982
6	OCH_2CH_2O	$N(C_4H_9)_2$	83	viscous oil	-	Delaney *et al*., 1969
7	OCH_2CH_2O	NHC_6H_5	39	149 - 150	-	Ahluwala *et al*., 1931
8	OCH_2CH_2O		68	146 - 147	146	Koul *et al*., 2000
9	OCH_2CH_2O		40	156 - 157	160	Venkatasamy *et al*., 2004
10	OCH_2CH_2O		44	88 - 89	89	Koul *et al*., 2000
11	H		51	115 - 116	122	Cromwell and Caughlan, 1945

Table II. LD_{50} (μg mg^{-1} larvae) of amides inhibiting the growth of *Spodoptera frugiperda* larvae (second instar) administered topically.

Amide*	Slope ± SE	LD_{50} (CI 90%)
1	0.18 ± 0.06	388.8 (47.37–836.9)
6	0.23 ± 0.087	504.07 (42.9–851.1)
8	0.24 ± 0.085	14.14 (2.93–580.65)
10	0.68 ± 0.063	1.07 (0.12–5.2)
11	0.54 ± 0.105	17.07 (6.55–74.86)
Piperine	0.58 ± 0.073	41.79 (22.6–86.9)

SE, standard error; LD, lethal dose; CI, confidence interval; N = 300 insects.
* Amides **2**, **3**, **4**, **5**, **7** and **9** were tested but the mortality did not differ from control (1%).

concentration of the active compound would take the great variations in mortality of the larvae, *i.e.*, would result in a homogeneous response of the population to the compound. On the other hand, low slope values, with great variations in the concentration of the active compound, would result in small variations in mortality, *i.e.*, the population would answer of heterogeneous form (Estrela *et al.*, 2003).

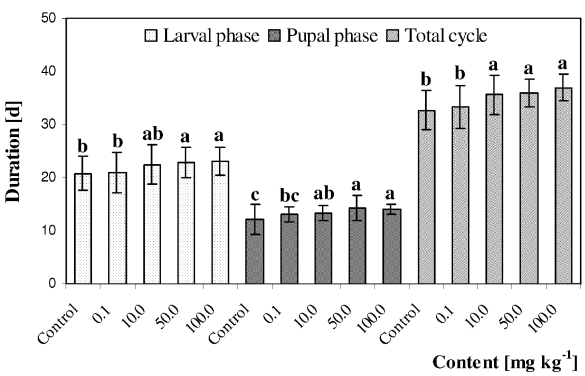

Fig. 2. Mean duration (days) of the larval and pupal phases and total cycle (from larvae to emergenc of the adult) of *S. frugiperda* with amide **10** administered in the diet. Means followed by the same letters indicate no significant difference ($P \leq 0.05$) in the Tukey test.

Fig. 2 presents the results of the ingestion bioassay as mean duration (days) of larval and pupal phases and total cycle (from larvae to emergence of the adult) of *S. frugiperda* with amide **10** administered in the artificial diet. It was observed that there was statistical difference between the treat-

ments and the control, in the larval and pupal phases and consequently in total cycle. Larvae phase control took (20.8 ± 3.2) days to reach the pupation, whilst those diet containing 100 and 50 mg kg^{-1} of amide **10** took (23.1 ± 2.6) and (22.9 ± 2.9) days, respectively, therefore it was verified a slow larval development. There was also an increase in pupal phase from (12.1 ± 2.9) (control) to (14.1 ± 1.0) days (100 mg kg^{-1} amide **10**). A small but significant prolongation of the larval and pupal phases of the fall armyworm produced a statistically significant increase in the total cycle duration time of 4.3 days.

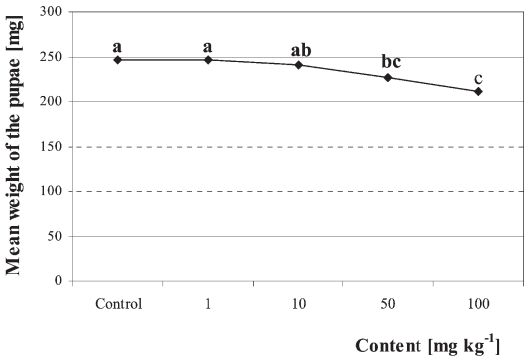

Fig. 3. Mean weight of pupae (mg) of *S. frugiperda* with amide **10** administered in the diet. Means followed by the same letters indicate no significant difference ($P \leq 0.05$) in the Tukey test.

Significant differences were observed for pupae weight resulting from the larvae fed on diet containing amide **10** (Fig. 3). At contents of 100 and 50 mg kg^{-1} it caused the highest reduction in pupae weight; at 10 mg kg^{-1} the reduction was moderate. However, there was no statistical difference for the pupal weight at the content of 1.0 mg kg^{-1}, when compared with the control. Thus, mean pupal weight varied from 246.4 mg (control) to 211.4 mg (100 mg kg^{-1}), *i.e.*, by 35 mg.

It was verified that amide **10** was active by ingestion, corroborating the results obtained by Scott and McKibben (1978) and Gbewonyo *et al.* (1993) for the *Piper* sp. extracts against several stored grain insects. When analyzing the effect of amide **10** over the life cycle of the fall armyworm, we verified that increasing contents of amide in artificial diet caused both a prolongation of the larval and pupal phases and a decrease in weight of the pupae, consequently, an increase in the development time since there was an enhancement in the time for pupation and emergence of adult. Similar results were also obtained by Dyer *et al.* (2003) employing a mixture of three natural amides from *P. cenocladum*.

The prolongation of the larval and pupal phases associated with a decrease in weight of pupae indicates inhibition in both growth and food consumption and suggests also low efficiency in dietary conversion (Tanzubil and McCaffery, 1990). This reduction in growth indicates two possible modes of action, feeding deterrence causing a reduction in food consumption and/or post-ingestive chronic toxicity (Wheeler *et al.*, 2001). As a consequence of these results, the insect could be more vulnerable to the action of entomopathogens, entomophagous agents and environmental variations, since it remains longer in the field (crop) (Tanzubil and McCaffery, 1990; Batista-Pereira *et al.*, 2002). Moreover, adults emerging from low-weight pupae could be more debilitated and would have lower capacity of competition for vital activities than individuals from healthy pupae. The longer development time might also benefit IPM (integrate pest management) strategies by reducing the number of generations of fall armyworm per season because of the asynchronization (Mikolajczak *et al.*, 1989).

The mean mortality can be seen in Fig. 4. There were significant differences among the contents of amide **10** with respect to mortality of larval and pupal phases and total cycle. The mortalities ranged from 16% (control and 1.0 mg kg^{-1}) to 42% (10, 50 and 100 mg kg^{-1}) for the larval phase, from 10% (control) to 35% (10, 50 and 100 mg kg^{-1}) for the pupal phase. Therefore it was verified that amide **10** caused mortality from 10 and 1 mg kg^{-1} content for the larval and pupal phases, respectively, reaching maximum mortality in both at 100 mg kg^{-1}. These variations interfered significantly with the total cycle mortality, the observed average mortality varying from 26% (control) to 72% (100 mg kg^{-1}). These results indicate that *S. frugiperda* is more sensitive to amide **10** during the larval phase, although in the pupal phase has occurred significant mortality resulting from the action of the amide in the larval phase.

Contact bioassays in addition with the results of the ingestion bioassay suggest that the modes of action of amide **10** can be by contact toxicity, feeding deterrence and/or post-ingestive chronic toxicity.

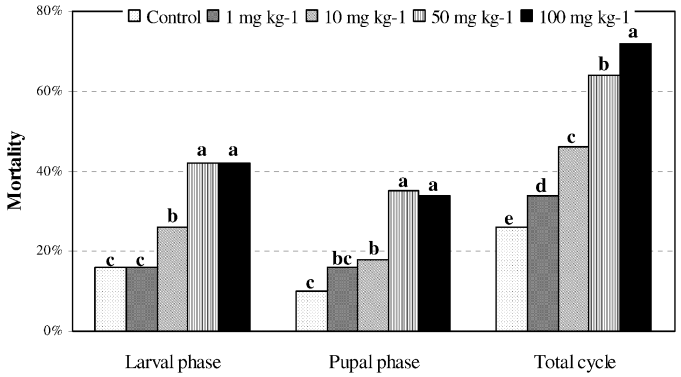

Fig. 4. Mortality (%) of the larval and pupal phases and total cycle of *S. frugiperda* with amide **10** administered in the diet. Means followed by the same letters indicate no significant difference ($P \leq 0.05$) in the Tukey test.

Structure-activity relationships indicate that the substituents in the amino group and in the aromatic ring might play a crucial role in the insecticidal activity. Although, we have verified a low to moderate toxicity effect for amides **1**–**9** and **11** (Table I), Neal (1989) has described that fagaramide, a natural isomer of amide **1**, is a phytosynergist, a plant compound that is present at concentrations producing no toxic effect by itself but has a synergistic effect on co-occurring toxins. Fagaramide has shown a synergistic effect on the toxicity of xanthotoxin to *Heliothis zea* (Lepidoptera: Noctuidae). Thus, this class of compounds merits further studies as potential control agents or as lead compounds. To improve the activity new derivatives will be synthesized.

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