BIOLOGICAL ACTIVITY OF THREE ALKYL CINNAMATES ON YOUNG LARVAE OF *TUTA ABSOLUTA*

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SUMMARY

The "tomato moth," *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a neotropical oligophagous insect considered a key pest of tomato crops. It was detected in Europe seven years ago and quickly spread to several regions of Asia and North Africa. In Argentina, its control is done by chemical pesticides mainly. The indiscriminate use of chemical broad spectrum pesticides have caused several problems in the control of this pest due to resistance mechanism involved to several insecticides including pyrethroids, organophosphates and biopesticides. In this context, the searching and evaluation of new compounds- compatible with integrated pest management programs- has become relevant.
Within the phenylpropanoids, alkyl cinnamates, whether natural or synthetic, have been reported with biological activity: repellence, antifeedant and insecticide. Therefore, the cinnamates could be a valuable alternative to replace the conventional insecticides. The aim of this work was to study the antifeedant effect of three alkyl cinnamates (methyl, ethyl and propyl cinnamate) on the consumption rate patterns of second instar larvae of *T. absoluta*. A series of concentrations of each compound (100, 250 and 500 micrograms/milliliter) were prepared using acetone (analytical grade) - distilled water as solvent and a surfactant (Tween 80®) was added to each solution to improve the wet of leaves. Tomato leaf disks of 2 cm diameter were treated by immersion in each solution during 15 seconds. Afterward, the treated discs were dried under fume hood and each disk was placed in a plastic capsule. A larva with 6-8 hours of starvation was added to each experimental unit. Each treatment was replicated between 24-30 times. The area consumed by each larva was measured after 24, 48 and 72 hours post-treatment. We also evaluated development time, weight of the pupa, adult emergence, fecundity and fertility as sublethal effects. The results were analyzed using ANOVA test.
None of the compounds tested at the aforementioned concentrations exhibited insecticidal effect. However, ethyl cinnamate showed a strong antifeedant effect. The results observed on the leaf consumption and other sublethal effects assessed will be discussed. According to these preliminary results, further studies are needed to complete its toxicological profile by other exposure methods.

INTRODUCTION

The "tomato moth," *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a neotropical oligophagous insect considered a key pest of tomato crops (*Lycopersicon esculentum* Mill.). It was detected in Europe in 2006 and quickly spread to several regions of Asia and North Africa (Desneux *et al*., 2010). Its life-cycle comprises a series of development stages: egg, four larvae stages, pupa and adult. The larvae feed on the mesophyll of leaves, stems and fruits of tomato creating conspicuous mines and galleries, reducing the photosynthetic capacity of the plant and favoring the invasion of the galleries in stems and fruits by secondary pathogens.
The Integrated Pest Management (IPM) is based on the integration and implementation of different pest control strategies (biological, chemical, cultural and physical ones) that are en-

vironmentally, economically and socially viable (Shenk and Kogan, 2003). However, in Argentine *T. absoluta* control is done by chemical pesticides mainly. The indiscriminated use of broad spectrum chemical pesticides has caused several environmental problems and failures in control due to insect's resistance mechanisms involved towards several insecticides including pyrethroids, organophosphates and abamectin (Lietti *et al*., 2005). In this context, the search and evaluation of new compounds, compatible with IPM programs has become relevant.

Within the phenylpropanoids, alkyl cinnamates, whether natural or synthetic, have been reported with biological activity: repellence, antifeedant and insecticide. Specifically, methyl cinnamate was shown to have insecticide effect against adults of *Sitophylus oryzae* (Coleoptera: Curculionidae) (Lee *et al*., 2008) and *Musca domestica* (Diptera: Muscidae) (Peterson *et al*. 2000) whereas ethyl cinnamate had antifeedant effect against *Spodoptera littoralis* (Lepidoptera: Noctuidae) (Abdelgaleil *et al*., 2008) and *Hylobius abietis* (Coleoptera: Curculionidae) (Sunnerheim *et al*., 2006). Moreover, it showed insecticide effect against larvae of *Culex pipiens pallens* (Diptera: Culicidae) (Kim *et al*., 2008) and *S. littoralis*. In the case of propyl cinnamate, it showed insecticidal effect on *M. domestica* adults (Peterson *et al*., 2000). Therefore, the cinnamates could be an alternative to replace the conventional insecticides.

The aim of this work was to study the antifeedant effect of three alkyl cinnamates (methyl, ethyl and propyl cinnamate) on the consumption rate patterns of second instar larvae of *T. absoluta.* Besides, the development time of immature stages, pupae weight, adult emergence, fecundity and fertility were evaluated as sublethal effects.

MATERIALS AND METHODS

Insect rearing

Plant material with larvae of *T. absoluta* was collected in fields of the Cinturón Hortícola Platense (La Plata, Argentina) in crops without any history of pesticides. The material collected was maintained under quarantine for discarding parasitism. After this period, insects were reared in the laboratory under controlled conditions: 25°C ±2°C, 70% ± 5% RH and a 14:10 (L: D) photoperiod. Larvae were fed tomato seedling of commercial variety whereas adults were fed 15% honey solution.

Chemicals

Ethyl *trans*-cinnamate *(99% w/w)* and methyl *trans*-cinnamate *(99% w/w)* were purchased from Sigma – Aldrich chemical company and were used without purification. Propyl cinnamate was prepared by a standard procedure: in a 50 ml round-bottomed flask, fitted with both a humidity-protected reflux condenser, and a device for absorbing evolved ClH gas, were placed 1.50 ml (20 mmol) of dry 1-propanol and 3.65 g (22 mmol) cinnamoyl chloride (Aldrich). The mixture was refluxed by 2 hours at 110ºC with stirring and then was cooled. The reaction mixture was washed with aq. 5% $NaHCO_3$ and with water, and then dried with anhydrous magnesium sulfate. The crude product was cautionly distilled, yielding 3.25 g (72%) of the ester, b. p. 131°C/10 torr. Acetone pro- analysis (Biopack) was used as solvent.

Bioassays

A series of concentrations of each compound (100, 250 and 500 µg/ml) were prepared by dissolving in 1 ml of acetone and 99 ml of distilled water. Each solution was added 0.01 ml of Tween 80® as surfactant. Solution control was performed with 1 ml of acetone, 99 ml of distilled water and 0.01 ml of Tween 80®. Tomato leaf disks of 2 cm diameter were prepared from mature tomato leaves, which were extracted from cultures without exposure to pesticide. Each disk was treated by immersion in the corresponding solution during 15 seconds. Afterward, the treated discs were dried under fume hood and each disk was placed on a square of moist filter paper in a transparent plastic capsule of 8 centimeter of diameter for 2 centimeter of height. A larva with 6-8 hours of starvation was added to each capsule (experimental unit). Each treatment was replicated between 24-30 times. The bioassays were carried out in the laboratory under the controlled conditions as above mentioned.
The discs were scanned at 24, 48 and 72 hours post-treatment and consumed area was measured using ImageJ program. At 96 hours one untreated tomato leaf was added to each experimental unit.
The development time of immature stages, weight of pupae, adult emergence, fecundity and fertility of females were analyzed as sublethal effects. For fecundity and fertility evaluation, 5-days-old females mated were isolated in transparent plastic containers of 10 cm diameter and 17 cm height, covered by cloth voile with a tomato leaf and oviposition was recorded daily and during five consecutive days.

Statistical analysis

The consumed area in different treatments, fecundity and fertility were analyzed by one-way ANOVA test and the means were compared by LSD test, using the program Statgraphic Version 5.0. The percentage of feeding inhibition was calculated from the formula: Antifeedant Inhibition Index (AII%) = [(1- T/C) x 100], where T is the average consumption of treated food and C is the average consumption of untreated food (Rosetti *et al*., 2008). The relationship between development time, pupal weight, adult emergence and area consumed was analyzed by correlation analysis using Statistica Version 7.0 program.

RESULTS

As shown in Figure 1, the consumed area differed significantly between the different treatments at 24, 48 and 72 hours ($F: 27.33$, $df: 235\text{-}9$, $p < 0.00001$, $F: 12.72$, $df: 234\text{-}9$, $p < 0.00001$, $F: 17.40$, $df: 232\text{-}9$, $p < 0.00001$, respectively). The main groups, according to LSD test, indicate that the area consumed was higher (attractant effect) for the ethyl cinnamate al concentration of 100 µg/ml and was lowest (dissuasive effect) for the same compound at concentrations of 250 and 500 µg/ml and for the propyl cinnamate at concentration of 500 µg/ml (Figure 2). The antifeedant inhibition index at 24 hours for the ethyl cinnamate at 250 and 500 µg/ml was 72 and 73%, respectively, and 76% for the propyl cinnamate at 500 µg/ml. Propyl cinnamate at concentration of 500 µg/ml had a phytotoxic effect. Methyl cinnamate at three concentrations has showed no effect on leaf consumption. None of the compounds tested at the aforementioned concentrations exhibited insecticidal effect.
Relationship were found between consumed area, immature stages development time and adult emergence ($r=0.28$ negative; $r= 0.30$ negative respectively). No significant differences were found between area consumed and pupal weight.

None of the compound evaluated showed any detrimental effect on the fecundity and fertility of females.

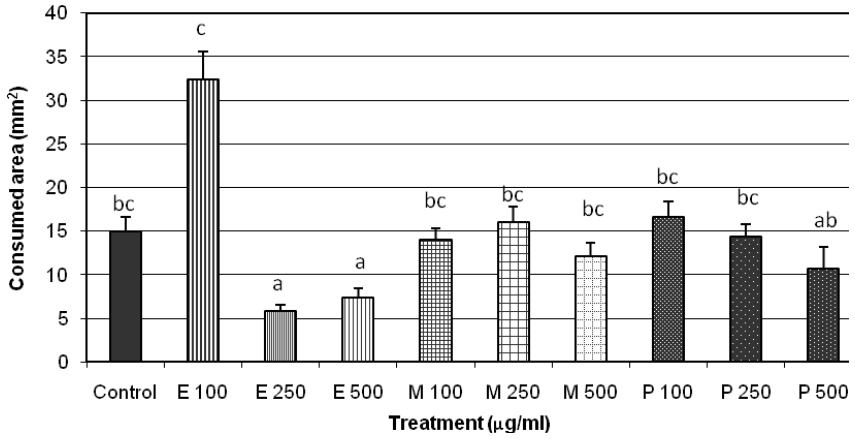

Figure 1. Treatment vs. Consumed area at 72 hours

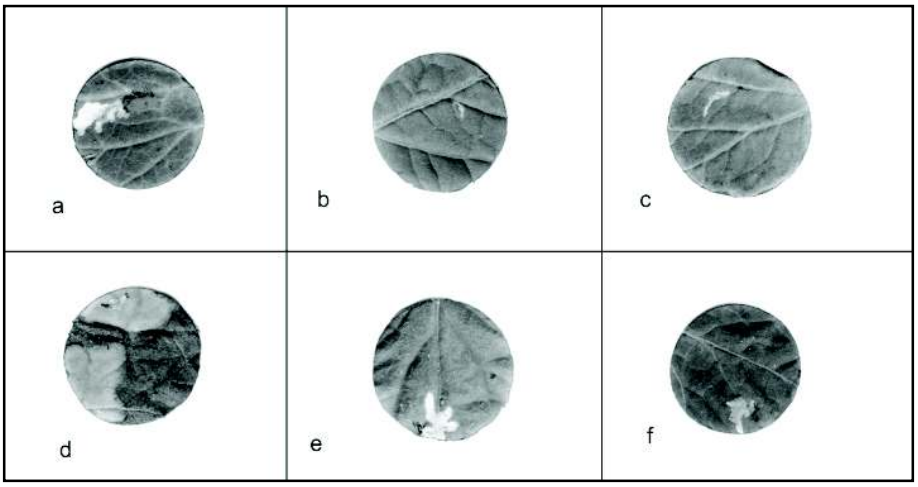

Figure 2. Treated and control discs at 48 hours.

a. Ethyl cinnamate 100 µg/ml
b. Ethyl cinnamate 250 µg/ml
c. Ethyl cinnamate 500 µg/ml
d. Propyl cinnamate 500 µg/ml
e. Methyl cinnamate 500 µg/ml
f. Control

DISCUSSION

Ethyl *trans*-cinnamate revealed a strong antifeedant activity at concentrations of 250 and 500 µg/ml (Antifeedant Inhibition Index= 72 and 73%, respectively) when evaluated by no-choice test against the second instar larvae of *T. absoluta*. However, it had attractant effect at concentration of 100 µg/ml. These results differ from those reported for the same compound on third instar larvae of *Spodoptera littoralis* in which there was an AII% = 72.1% for the concentration of 100 µg/ml (Abdelgaleil *et al*., 2008).

Antifeedant activity of propyl cinnamate at concentration of 500 µg/ml could be related to the phytotoxicity observed.

Development time was longer when consumption was lower. However, no relation was found between the consumed area and pupae weight probably because larvae could recover their weight rapidly by providing untreated tomato leaves from 96 hours post-treatment.

According to these preliminary results, further studies are needed to complete the toxicological profile of alkyl cinnamates compounds by other exposure methods.

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