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Biological effects of *Physalis peruviana* L. (Solanaceae) crude extracts and its major withanolides on *Ceratitis capitata* Wiedemann (Diptera: Tephritidae)

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Biological effects of *Physalis peruviana* crude extracts and its major withanolides (withanolide E and 4-ß-hydroxywithanolide E) were investigated on larvae and adults of the fruit fly *Ceratitis capitata*. High concentrations of crude extracts (10000 and 35000 ppm) in larval diet caused 100% mortality while low concentration (1000 ppm) caused significative differences in larval mortality, development delay and puparia length. Withanolide E and 4-ß-hydroxywithanolide E (500 ppm) also produced significative mortality on larvae. The application of crude extracts to adults drinking vessels caused significative lethal effects at 10000 and 35000 ppm. These data indicate that *P. peruviana* crude extracts and its two major withanolides could be used to develop baits to control *C.capitata*.

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Key words: secondary metabolites, natural insecticides, mortality, development alterations.

INTRODUCTION

The study of allelochemical interactions among insects and plants is currently one of the most actively investigated subjects in chemical ecology, partly due to its interesting prospective for development of new biorational pesticides of natural origin (SCHOONHOVEN & VAN LOON, 1998). These interactions involve numerous secondary plant metabolites that may interfere with the behavior, growth, or development of the insects. It has been observed for a group of specialized metabolites, the withanolides, isolated from several Solanaceae species (RAY & GUPTA, 1994). Some of them exhibit activity as feeding deterrents (ASCHER *et al.*, 1987) or ecdysteroid antagonists (DINAN *et al.*, 1996), and they have been related to chemical defense mechanisms (BAUMANN *et al.*, 1993).

Physalis peruviana Linnaeus or "cape gooseberry" is a member of the Solanaceae characterized by the presence of two major components: withanolide E (**1**) and 4-ß-hydroxywithanolide E (**2**) (TOMASSINI *et al.*, 2000).

This plant was previously reported as one of the first Solanaceae that showed antifeedant properties in biological assays (ASCHER

et al., 1980; ELLIGER *et al*., 1994). BAUMANN & MEIER (1993) determined the importance of its major withanolides as a chemical defense against herbivory during fruit development. WAISS *et al.* (1993) studied the effect of *P. peruviana* withanolides on the growth and development of *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), an economic important pest of some Solanaceae crops.

In this work we studied the biological effects of crude extracts from *Physalis peruviana* and its major compounds, withanolide E (**1**) and 4-ß-hydroxywithanolide E (**2**) against the mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), an economically important pest, to determine mortality, development delays and reduction in surviving adult size.

MATERIALS AND METHODS

Insects

Bioassays were conducted on *Ceratitis capitata* larvae and adults (Diptera: Tephritidae) obtained from an established laboratory colony. Larvae were maintained at 25 ± 2 °C and 70 ± 5% relative humidity in darkness on an artificial diet (BADO *et al.* 2004). Adults were reared on a sugar and beer yeast (3:1) diet (VARGAS, 1989), water being provided in 20 ml plastic vessels. Artificial fruits were used for oviposition (TERÁN, 1977).

Test Compounds

Aerial parts of *P. peruviana* were collected from a crop held in Agronomy Faculty of Buenos Aires, Argentina. The dried and pulverized aerial parts of *P. peruviana* (730 g) were triturated and macerated successively with ether (1ml/g of plant, 3 days) and ethanol (1ml/g of plant, 3 days) at room temperature. The residues obtained after evaporation of the combined extracts (3.65 g) were initially fractionated by vacuum liquid chromatography using hexane-EtOAc mixtures of increasing polarity (100:0-0:100) as eluant. The fraction eluted with EtOAc was purified by flash chromatography yielding to withanolide E (432.5 mg). The same purification on fraction eluted with hexane-EtOAc 20:80 and yield to 4β-hidroxiwithanolide E (48.3 mg). The compounds withanolide E and 4ß-hidroxiwithanolide E (Figure 1) had 1H and 13C NMR spectra identical to those previously described. Prior to biological testing, all compounds were analyzed by TLC on silica gel 60 F254 (Merck) pla-

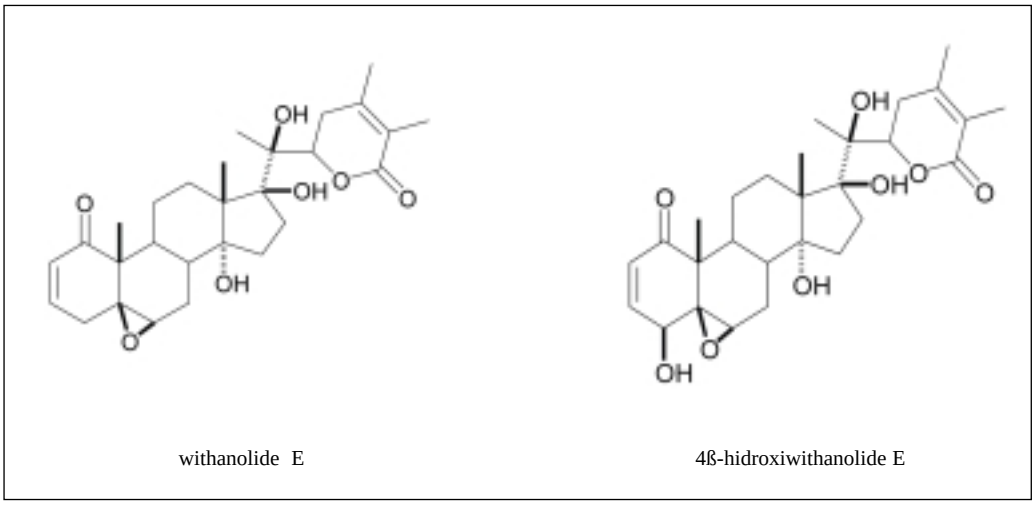

Figure 1.Chemical structure of withanolides assayed.

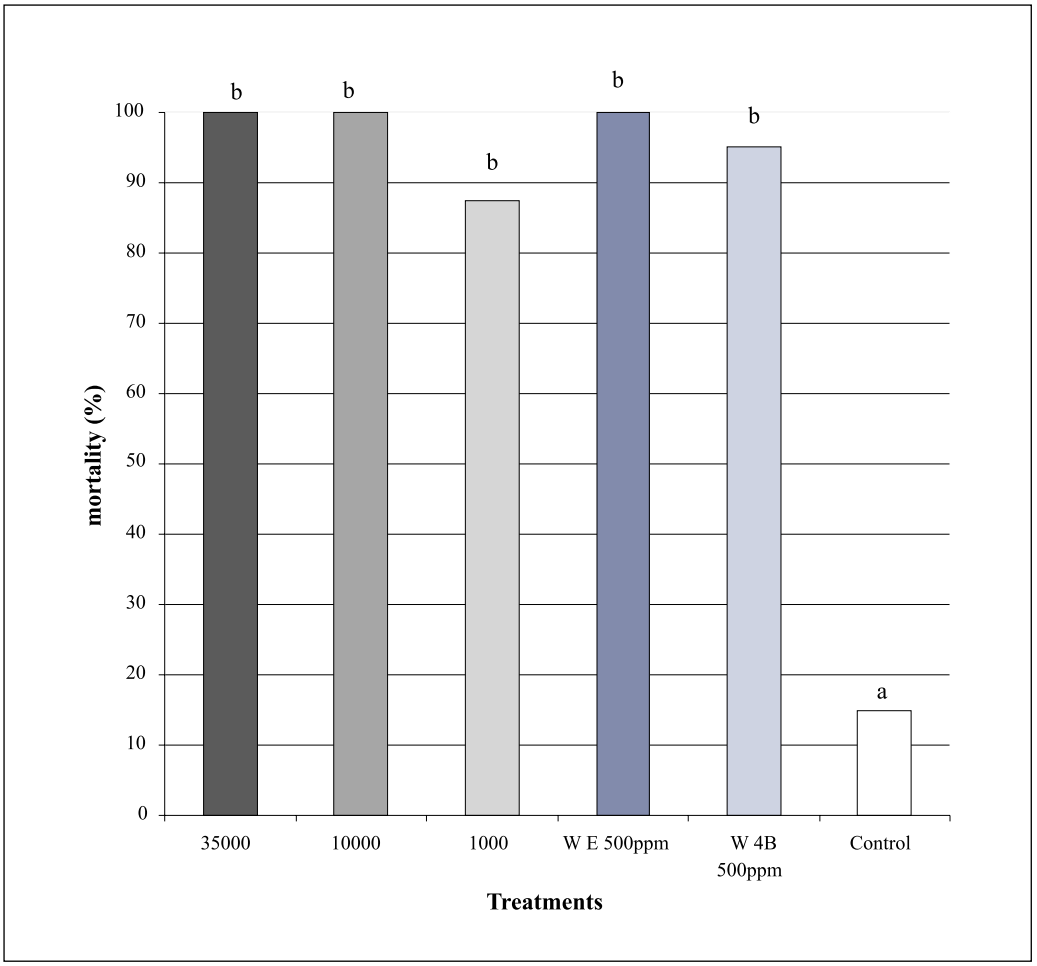

Figure 2. Mortality (%) of *C. capitata* individuals exposed to artificial diet treated with *P. peruviana* crude extracts (1000, 10000 and 35000 ppm), withanolide E and 4-ß-hydroxywithanolide E (500 ppm). Different letters at the top of the bars indicate significative differences ($p \leq 0.05$).

tes using hexane/EtOAc mixtures as mobile phase. Spots were visualized by spraying 10% H2SO4 in EtOH and heating. A purity of >95%, as verified by 1H NMR spectroscopy, was considered to be acceptable.

Bioassays

Activity on Larvae

Groups of 10 neonatae larvae were reared in plastic vessels (4.5 cm height, 4.5 cm diameter) containing artificial diet (TERÁN, 1977), where compounds were previously incorporated. Crude extracts were dissolved in ethanol in order to reach three concentrations: 35000, 10000 and 1000 ppm, while pure withanolides (withanolide E and 4-ß-hydroxywithanolide E) were evaluated at 500 ppm. Control larvae were reared in artificial diet where only ethanol was added. Plastic vessels containing larvae were kept inside plastic cylinders (10 cm height, 8.5 cm diameter) with sterilized sand as puparia

medium and held under standardized conditions (25 ± 2°C and 70 ± 5% RH, in darkness). The puparia number was daily recorded. After puparia length was registered, they were transferred to glass cylinders (7 cm height, 4.5 cm diameter) to record adult number. Mortality from neonatae larvae to adult emergence (percent) was calculated. Four replicates of each treatment were assayed.

Activity on adults

Crude extracts were suspended in pure water containing a drop per milliliter of Tween 20 as a tensioactive, to obtain final concentrations of 35000, 10000 and 1000 ppm. Suspensions were added to 20 ml plastic drinking vessels containing 5 mm diameter glass balls to avoid adults immersion (BUDIA *et al*., 1988). Controls received only water with Tween 20. Each drinking vessel was placed inside a glass cylinder (10 cm height; 8.5 cm diameter) together with small plates containing 10 g of beer yeast and sugar (1:3) as a food source. Groups of 10 newly hatched adults were released inside each cylinder. Every 48 h (three times during the bioassays) fresh stock suspensions were added to the drinking vessels. Mortality was recorded daily until day seven. Four replicates of each treatment were assayed.

Statistical Analysis

Activity on larvae. Adults number, expressed as a percentage in relation to the number of exposed larvae, was used to calculate by Probit analysis (LITCHFIELD &

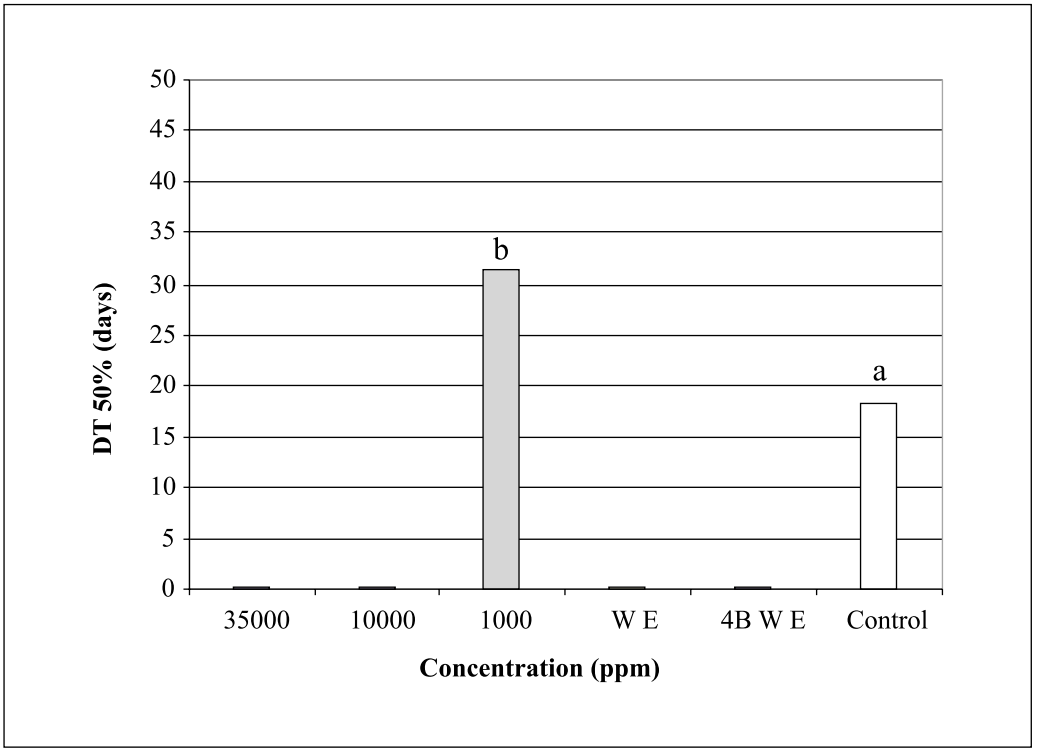

Figure 3. Development time 50% (days) of *C. capitata* larvae exposed to crude extracts (1000, 10000 and 35000 ppm), withanolide E and 4-ß-hydroxywithanolide E (500 ppm) from *P. peruviana*. Different letters at the top of the bars indicate significative differences ($p \leq 0.05$).

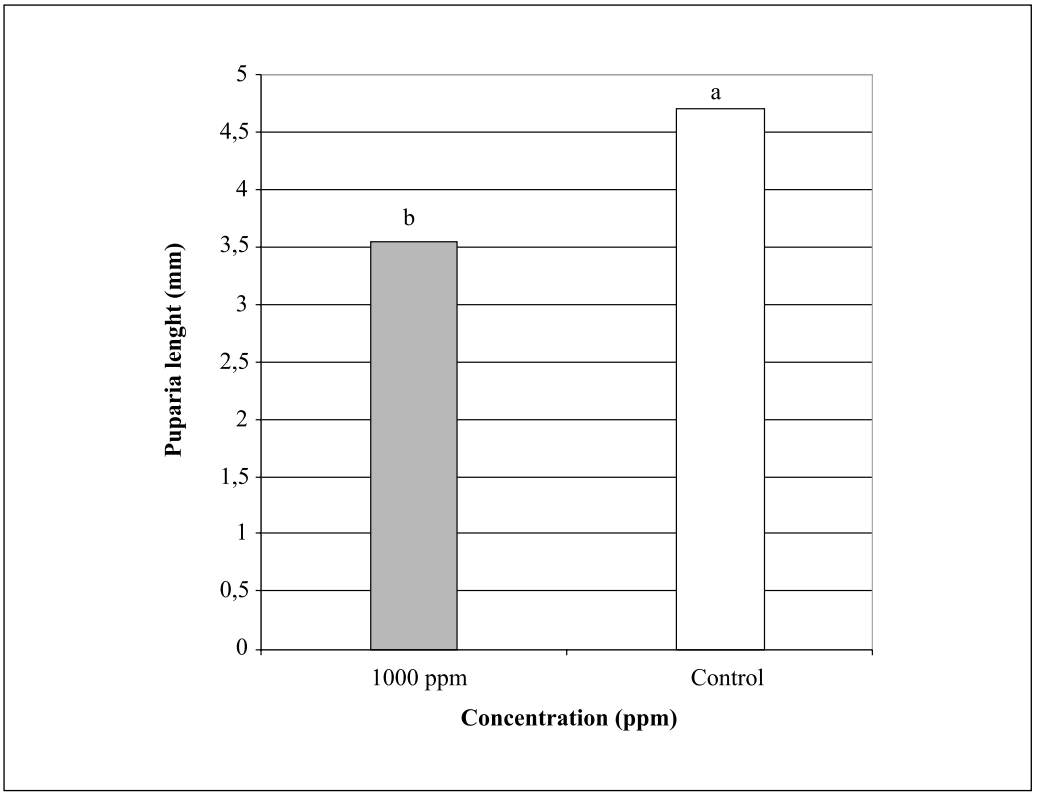

Figure 4. Puparia lenght (mm) of *C.capitata* individuals reared in artificial diet containing *P. peruviana* crude extracts at 1000 ppm and controls. Different letters at the top of the bars indicate significative differences ($p \leq 0.05$).

WILCOXON, 1949) the parameter DT_{50} (development time 50%: time needed for 50% of exposed larvae to reach adult stage).

Significant development delays were determined by no superposition of confidence limits between DT_{50} of treatments and controls. ANOVA and Tukey´s multiple-range test were used for mortality and puparia length data ($p \leq 0.05$).

Activity on adults. Mortality data were analyzed with ANOVA and Tukey´s multiple-range test ($p \leq 0.05$)

RESULTS AND DISCUSSION

Figure 2 shows that, when crude extracts and both withanolides were applied to larval diet, the two higher concentrations (35000 and 10000 ppm) produced 100% mortality of larvae while the lowest concentration (1000 ppm) caused 87.5% mortality. The remaining 12.5% of individuals resulted in puparia significantly smaller (Figure 4). At this concentrations individuals reached the adult stage in a period significantly longer than controls (Figure 3), showing considerable development delays (DT_{50}). In the case of 10000 and 35000 ppm treatments, DT_{50} couldn't be calculated because 100% mortality occurred previously to the puparia stage (Fig. 3). For the same reason, puparia length was not registered (Fig. 4).

When adults were exposed to crude *P. peruviana* extracts incorporated in drinking vessels, the two higher concentrations

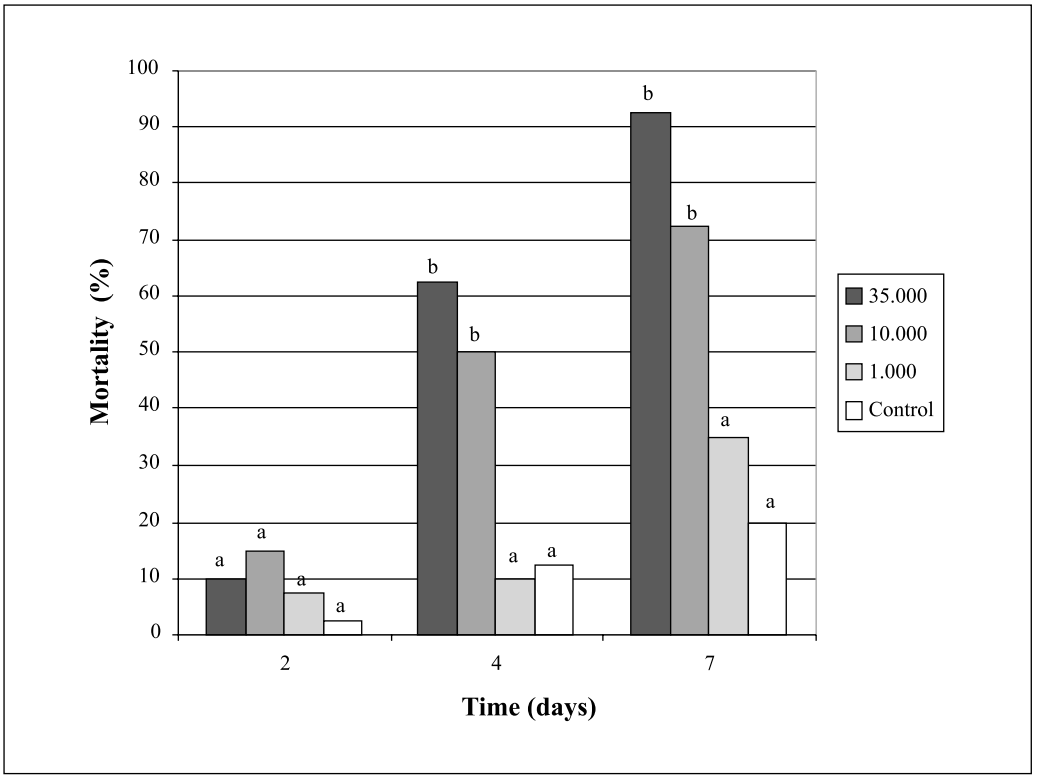

Figure 5. Mortality (%) of *C. capitata* adults exposed to *Physalis peruviana* crude extracts in drinking vessels. Different letters at the top of the bars indicate significative differences ($p \leq 0.05$).

(35.000 and 10.000 ppm) caused significant mortality (Figure 5) after four days of exposure, lethal effect which increased after seven days of exposure. The 1.000 ppm concentration didn't produce any biological effect.

The difference here observed between the lethal effect of 1.000 ppm treatment to larvae (Fig. 2) and no lethal effect to adults (Figure 5) could be attributed to the fact that the deep transformations experimented by holometabolous insects during pupation stage, make the juvenil instars more susceptible than the adults to the presence of xenobiotic compounds (WIGGLESWORTH, 1972).

When the two major withanolides (withanolides E y 4-β-hydroxywithanolide E) were applied to the diet, their effect varied according to the compound (Fig. 2). Withanolide E produced 100% larval mortality while 4-β-hydroxywithanolide E caused 92,5% larval mortality. This high lethal effect avoid that many individuals survive until puparia stage. This fact avoided the calculation of DT_{50} for these compounds (Fig. 3). Lethal effects here observed are similar to those shown by ELLIGER & WAISS (1989) who calculated EC_{50}=250 ppm for 4-β-hydroxywithanolide E with the lepidopteran *Helicoverpa zea.* Both compounds have shown sublethal effects on other important agricultural pests. Lower concentrations of withanolide E and 4-ß- hydroxywithanolide (10 and 100 ppm respectively) reduced the ingestion of poliestiren lamellae of *Spodoptera littoralis* Boisd larvae (Lepidoptera) (ASCHER *et al.*, 1980)

Figure 6. *C. capitata* larvae reared in Terán (1977) artificial diet containing *P. peruviana* crude extracts at 1000 ppm.

meanwhile 500 ppm and 250 ppm concentrations resulted in no weight gain of *Epilachna varivestis* Mulsant larvae (Coleoptera) incorporated in bean leaves. BAUMANN & MEIER (1993) mentioned that different contents of withanolide E (640 ppm) and 4-β-hydroxywithanolide E (1400 ppm) protect the plant against predation.

This is the first report of lethal and sublethal effects of *P. peruviana* crude extracts and its major withanolides on *Ceratitis capitata* larvae. Lethal effects against adults could be considered an interesting property to be used in the elaboration of baits to control *C. capitata* adults in fruit trees. In this way, *P. peruviana* crops could not only provide edible fruits but could be a source of natural insecticides, too. Further studies in other pests are recommended.

RESUMEN

CIRIGLIANO, A., I. COLAMARINO, G. MAREGGIANI, S. BADO. 2008. Efectos biológicos de extractos crudos de *Physalis peruviana* L. (Solanaceae) y sus withanolidos mayoritarios sobre *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). *Bol. San. Veg. Plagas*, **34**: 509-515.

Se investigaron los efectos biológicos de extractos crudos de *Physalis peruviana* y de sus withanólidos mayoritarios (withanolide E and 4-ß-hydroxywithanolide E) sobre larvas y adultos de mosca de los frutos *Ceratitis capitata*. Concentraciones altas de extractos crudos en la dieta larval (10000 and 35000 ppm) produjeron mortalidad de 100% mientras que la concentración baja (1000 ppm) produjo diferencias significativas en mortalidad larval, demoras en desarrollo y en longitud de puparios. Los withanólidos E y 4-

ß-hydroxywithanólido E (500 ppm) también produjeron mortalidad larval significativa. La aplicación de extractos crudos en bebederos de adultos causó efectos letales a 10000 y 35000 ppm. Estos datos indican que los extractos crudos de *P. peruviana* y sus dos withanólidos mayoritarios podrían utilizarse para desarrollar cebos para el control de *C. capitata*.

Palabras clave: metabolitos secundarios, insecticidas naturales, mortalidad, alteraciones en desarrollo

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