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## Antifeedant and insecticidal activity of *Polygonum persicaria* extracts on *Nomophila indistinctalis*

[Actividad antialimentaria e insecticida a partir de extractos de *Polygonum persicaria* sobre *Nomophila indistinctalis*]

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### Abstract

**Context:** Vegetal extracts represent an alternative to control against agricultural pests that have become resistant to pesticides. Using natural products is considered to be more friendly to the environment and safe.

**Aims:** To determine the insecticidal and antifeedant activity of *Polygonum persicaria* extracts of two different populations in Chile (Valparaíso and Curico) against *Nomophila indistinctalis* larvae.

**Methods:** Insecticide Resistance Action Committee (IRAC) susceptibility test was used to evaluate the insecticidal activity of the extracts at concentrations of 100, 250, 500 and 1000 mg/L; against first instar larvae of *Nomophila indistinctalis*. The antifeedant activity was evaluated to determine the percentage of consumption in third instar larvae on treatment.

**Results:** When comparing the control and the treatment groups in the antifeedant activity assay, significant differences ( $p < 0.05$ ) were observed after 90 minutes of exposure. With respect to the insecticidal activity, all extracts showed significant effects at the applied concentrations compared to the negative control. Moreover, the dichloromethane extracts of Curico and Valparaíso at concentrations greater than 500 mg/L showed a similar insecticidal activity as compared to the commercial formulation Neem.

**Conclusions:** This work presents for the first time the results of the anti-feeding and insecticidal activity of ethanol, methanol, and dichloromethane extracts from *Polygonum persicaria* on *Nomophila indistinctalis*. The results show that the extracts of this species can be used as an alternative for biological control. In addition, the results obtained allow a bioguided fractionation for the identification of secondary metabolites present in these extracts.

**Keywords:** antifeedant activity; insecticidal activity; *Nomophila indistinctalis*; *Polygonum persicaria*.

### Resumen

**Contexto:** Los extractos vegetales representan una alternativa de control contra plagas agrícolas que se ha hecho resistentes a los plaguicidas. El uso de productos naturales es hoy en día más amigables con el medio ambiente y seguros como insecticidas naturales.

**Objetivos:** Evaluar la actividad insecticida y antialimentaria de extractos de *Polygonum persicaria* de dos poblaciones diferentes de Chile (Valparaíso y Curicó). Los extractos etanólicos, metanólicos y de diclorometano se evaluaron sobre larvas de *Nomophila indistinctalis*.

**Métodos:** Se usó el test de susceptibilidad de *Insecticide Resistance Action Committee* (IRAC) para evaluar la actividad insecticida de los extractos a concentraciones de 100, 250, 500 y 1000 mg/L; frente a larvas de primer estadio de *Nomophila indistinctalis*. Para evaluar la actividad antialimentaria se usó un método que determina el porcentaje de consumo de las larvas en tercer estadio sobre el tratamiento y el control.

**Resultados:** Al comparar el control y el tratamiento en la actividad antialimentaria sólo se observan diferencias significativas ( $p < 0.05$ ) en los tiempos superiores a 90 minutos de exposición. Acerca de la actividad insecticida, todos los extractos mostraron efectos significativos en las concentraciones aplicadas en comparación con el control negativo. Sin embargo, los extractos de diclorometano de Curicó y Valparaíso mostraron actividad insecticida similar a la formulación comercial Neem para concentraciones superiores a 500 mg/L.

**Conclusiones:** En este trabajo se presentan por primera vez los resultados de la actividad anti-alimentaria y actividad insecticida de los extractos de metanol, etanol y diclorometano de la especie *Polygonum persicaria* sobre *Nomophila indistinctalis*. Los resultados presentan que los extractos de esta especie pueden ser una alternativa de control biológico. Además, los resultados obtenidos permiten un estudio guiado por bioensayo de los metabolitos secundarios presentes en estos extractos.

**Palabras Clave:** actividad antialimentaria; actividad insecticida; *Nomophila indistinctalis*; *Polygonum persicaria*.

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## INTRODUCTION

The excessive use of synthetic insecticides on crops and their residues has generated resistance in pathogen populations making difficult to manage the pest control of many of them (Zettler et al., 2000; Isman, 2006; Karabelas et al., 2009; Kaushik et al., 2009). The research has been mainly focused on the development of more active and safe alternatives, and in this context, natural products as insecticides have been considered to be environmentally friendly and safe (Villaverde et al., 2016). Some studies have shown that extracts from plants have great potential in pest control, and this activity has been related to the content of different secondary metabolites (Ali et al., 2012; Razavi et al., 2015; Dutra et al., 2016).

The *Polygonum* genus (Polygonaceae) is constituted by about 300 species worldwide. The main secondary metabolites that have been reported from this genus are diterpenes, triterpenes, flavonoids, anthraquinones, coumarins, phenylpropanoid, tannins and stilbenoids (Smolarz, 2002; Derita et al., 2008; Derita and Zacchino, 2011a). Particularly in Chile, the family Polygonaceae is represented by seven genera and 40 species (Wilhelm de Moesbach, 1992). *Polygonum persicaria* is a perennial, morphologically variable and widely distributed plant. Stilbenes, flavones, flavonols, chalcones, flavonoids and phenolic acids have been found in this species (Smolarz, 2000; Derita and Zacchino, 2011b; Kurkina et al., 2013; Lajter et al., 2013). Moreover, antibacterial, antifungal and insecticidal activities have been reported for this species (Hussain et al., 2010).

The antifeedant and insecticidal activities from different plant extracts have been extensively studied in recent years (Taylor et al., 2004; Pavela, 2010; Sandoval-Mojica and Capinera, 2011; Muñoz et al., 2013). To the best of our knowledge, no similar studies have been realized with *Polygonum persicaria* growing in Chile.

*Nomophila indistinctalis* is a moth, which belongs to Crambidae family. Limited geographic distribution ranges have been described for this species. This lepidopteran is nocturnal and can cause damage in sunflowers, eucalyptus, beet and corn crops, among others (Zanuncio et al., 2001; Torreta et al., 2009).

In this paper, we report for the first time the antifeedant and insecticidal activities of the methanolic, ethanolic and dichloromethane extracts of *Polygonum* species against *Nomophila indistinctalis*.

## MATERIAL AND METHODS

### Chemicals

Nutrient agar GranuCulture™ (Darmstadt, Germany), Neem®X (Marketing Arm International Inc.), artificial diet Stonefly Heliothis (Ward's Science, New York), distilled water and other reagents were of analytical grade.

### Plant material

The aerial parts of *Polygonum persicaria* were collected during the month of February 2015 in two different regions of Chile: Tranque La Luz, Valparaíso Region, Valparaíso (33° 7' 7.23" S, 71° 34' 47.23" W), and Quechereguas, Maule Region, Curico (35° 6' 55.12" S, 71° 16' 53.15" W). The vegetal material was identified by Dr. Cristian Atala Bianchi from the Pontificia Universidad Católica de Valparaíso, and the samples of the plants were deposited in the herbarium of the Universidad de Concepción (CONC) Chile with Herbarium Number 183592, 183591, respectively.

### Preparation of the extracts

The aerial parts of *Polygonum persicaria* were dried at a temperature of 40°C for 72 h in a stove. Afterward, they were grounded and sieved obtaining a particle size of 38 mesh. Each sample was macerated with constant stir in 2 L of ethanol, methanol, and dichloromethane for 15 days. The extracts were filtered (qualitative Whatman filter paper, degree 1, thickness of 0.18 mm), concentrated under reduced pressure in a rotary evaporator (Büchi B-480, Büchi Labortechnik AG, Flawil, Switzerland) and stored in the darkness at a temperature of 4°C until analysis.

### Insects

Adult individuals of *Nomophila indistinctalis* were collected in the Panguilemo locality in the Maule Region, Talca (35° 22' 15" S, 71° 35' 50" W) in

December of 2014. For the identification of the species after experimental studies, abdominal tips were excised and their genitalia prepared for taxonomic identification. This analysis was realized by the entomology laboratory of Agriculture Service (S.A.G) of the city of Curico, Chile. The moths were taken to the laboratory and organized in different groups. The insects were fed with honey at 10% in water till the egg-laying. The hatched larvae were fed with an artificial diet Stonefly *Heliothis* in a controlled environment of  $22 \pm 1^\circ\text{C}$ , >65% relative humidity, with a photoperiod of (16 Light:8 Day) in a growth chamber (Downham et al., 2003).

### Determination of the antifeedant activity

The antifeedant activity against *N. indistinctalis* was studied using two disks of the leaf ( $1\text{ cm}^2$ ), one for the control (C) and the other for the treatment (T). The leaf disks were cut from healthy *Beta vulgaris* L. plants, lying in a 2% agar plate, placed in an equidistant form from the petri dish ( $9\text{ cm} \times 1\text{ cm}$ ). The disks (T) were covered with a 10  $\mu\text{L}$  of the extract, while the disks (C) were treated with 10  $\mu\text{L}$  of a solution of methanol and dichloromethane at 10%. The larvae *N. indistinctalis* in the third stage were individually assayed (20 repetitions). To measure the antifeedant activity, a visual punctuation of the consumed area was assigned to each disk (0, 12.5, 25, 37.5, 50, 62.5, 75, 87.5 or 100%) (Díaz et al., 2014).

### Determination of the insecticide activity

The extract effects were evaluated with first stages larvae of *N. indistinctalis* in different concentrations (100, 250, 500, 1000 mg/L). Briefly, 0.5 g (10-20 mm diameter) of artificial diet was placed in a 16-well multiplate. Then, a constant volume of the extract in different concentration was added to each dish. The commercial insecticide Neem (16 mg/L) and water were used as positive and negative controls, respectively. Then, 16 neonate larvae were analyzed in individual experiments in triplicate at each extract concentration. The number of dead larvae was counted after eight days of incubation at  $22 \pm 1^\circ\text{C}$ , >65% relative humidity, with a photoperiod of (14 Light: 10 Day) in a growth

chamber (Downham et al., 2003).

### Statistical analysis

For the antifeedant activity, the data were analyzed with one-way variance ANOVA and the differences between the treatments for every extract were established using the Tukey-HSD proof using the Statgraphics Centurion XV (Statpoint, Inc., VA, USA) software. Antifeedant (deterrent) activity determination was based on the proportions of the consumption of the leaf treated with solvent (control) compared to the proportions of the consumption of the leaf treated with the substance tested (treatment) (Díaz et al., 2014). The studied response in the insecticidal activity assay was the percentage of dead larvae in each determination. The observed differences in every extract concentration were analyzed using one-way ANOVA. All results were expressed as mean and standard error of the mean (SEM) values.

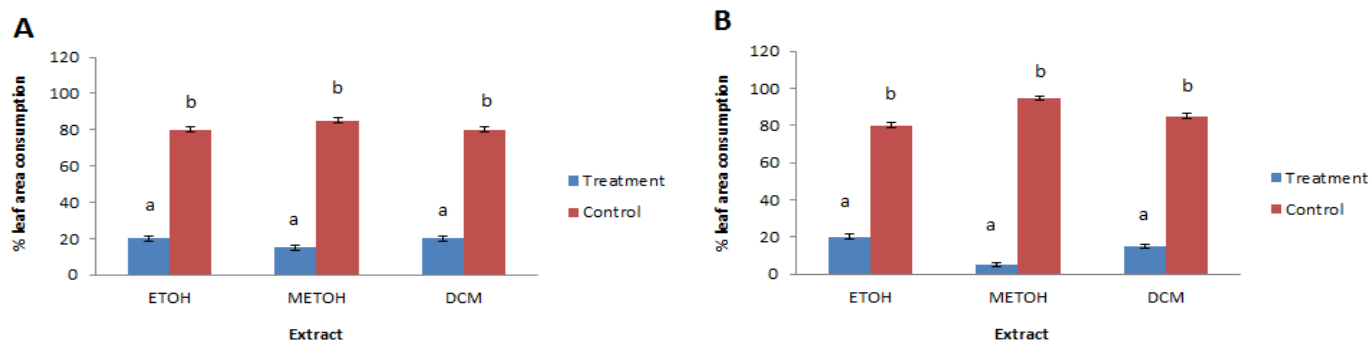
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## RESULTS

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### Plant material

The plants of *Polygonum persicaria* were collected in two different parts of Chile. One population of *P. persicaria* was found around the "Tranque La Luz" located in the Valparaiso Region. This plant reaches a height of 50 cm, with even and arrow-shaped leaves of 5 to 7 cm length. Additionally, the plant exposes clusters of red color emerging from the nodes of the plant. The other population of *P. persicaria* was collected in Curico (Quechereguas) above a water channel, presenting a typical height of 90 cm. The leaves and clusters had the same features of the ones collected in Valparaiso, despite the fact that the leaves were longer (10 to 13 cm length). *Polygonum persicaria* (Valparaiso) extracts were obtained by macerating dried parts with ethanol obtaining 1.9%, followed by methanol obtaining 7.1% and dichloromethane extract giving 0.3%. Using *Polygonum persicaria* (Curico) were obtained; 1.9% of ethanol, 7.4% of methanol and 0.3% of dichloromethane extract. The extracts from each locality showed no differences.



**Figure 1.** Percentage leaf area consumption of control and treatment (*P. persicaria* extracts) coming from Curico (A) and Valparaiso (B) by larvae of *N. indistinctalis* after 120 minutes of exposition.

Results expressed as mean  $\pm$  SEM, n=20. Different letters symbolize significant differences ( $p < 0.05$ ) by One-way ANOVA and Tukey HSD test.

### Antifeedant activity

Results observed in the present investigation revealed that in the antifeedant activity assay when comparing with the control, no significant differences ( $p > 0.05$ ) were found before 90 minutes of exposition. The Fig. 1 shows the percentage of consumption after 120 minutes of exposition.

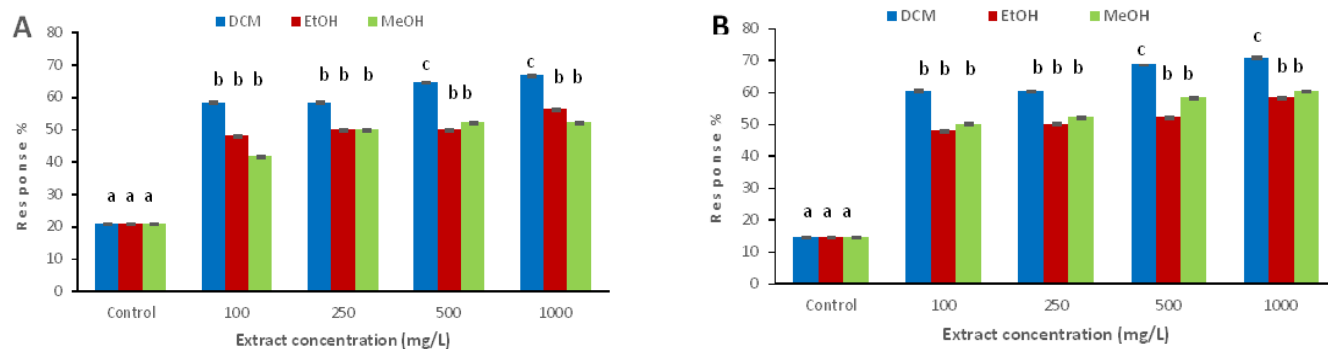
The comparative analysis of these results showed a significant difference between the control and the evaluated treatment ( $p < 0.05$ ), suggesting that the larvae prefer the control disks independently of the treatment evaluated. However, when comparing all the extracts, were observed small differences of the antifeedant activity increase in the treatment of methanol and dichloromethane of Curico with respect to the Valparaiso extracts.

### Insecticide activity

The insecticide effect of the different extracts of *P. persicaria* was evaluated at 100, 250, 500 and 1000 mg/L. The results are shown in Fig. 2, where it is specified the mortality registered for plants co-

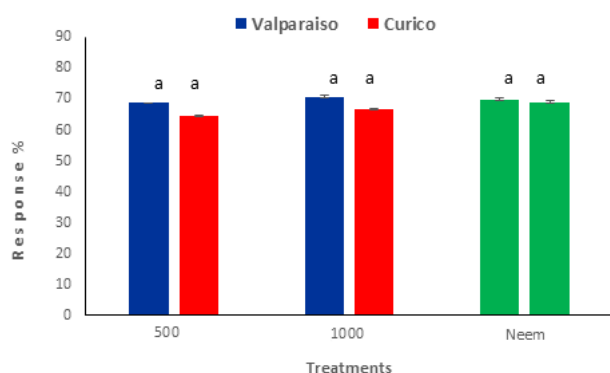
llected in Curico (Fig. 2A) and Valparaiso (Fig. 2B). Analyzing the data using one-way ANOVA, it was found that there were significant differences between the negative control and the treatments of all the extracts, independently of the origin ( $p < 0.05$ ). Although, it should be noticed that for concentration higher than 500 mg/L, the dichloromethane extract produced a higher mortality toll in the *N. indistinctalis* larvae when compared to the other extracts.

Considering the application of these extracts, the results of the dichloromethane extracts at 500 mg/L and 1000 mg/L were comparable with the commercial insecticide Neem at 16 mg/L (Fig. 3), which corresponds to the maximum value proposed by the product supplier for the control of species in larval or nymphal stage. Using the one-way ANOVA analysis, no significant differences between the extracts of *P. persicaria* from Curico and Valparaiso and the commercial insecticide were found (Tukey HSD test,  $p > 0.05$ ), this was detected in the Insecticide Resistance Action Committee (IRAC) susceptibility test.



**Figure 2.** Effects of the different concentration of *P. persicaria* extracts from Curico (A) and Valparaíso (B) in the insecticide assay (expressed as percentage of *N. indistinctalis* larvae death after eight days of exposition).

Results are expressed as mean  $\pm$  SEM, n=48. Different letters symbolize significant differences ( $p < 0.05$ ) by One-way ANOVA and Tukey HSD test.



**Figure 3.** Insecticide activity of the dichloromethane extracts obtained of *P. persicaria* collected from (A) Valparaíso and (B) Curico in concentrations of 500 and 1000 mg/L compared to a commercial product (Neem 16 mg/L).

Results are expressed as mean  $\pm$  SEM, n=48. Same letters symbolize no significant differences ( $p > 0.05$ ) by One-way ANOVA and Tukey HSD test.

## DISCUSSION

The results of the antifeedant activity could be explained by the presence of secondary metabolites like flavonoids, terpenes and other aromatic components that are usually present in some genus of *Polygonum* (Li and Lin, 1993; Xu et al., 2006). Some studies from the extracts obtained from *P. multiflori preparata* have described the presence of quercetin, rutin and kaempferol (Chen et al., 2000; 2001), which are known for their antifeedant and insecticide activity (Silva et al., 2003; Simmonds, 2003). Moreover, some components found in extracts of medium polarity like the rotenoids have shown feeding deterrence and insecticide effect (Gordon and Headrick, 2001). It has been reported that the presence of rotenoids in dichloromethane extracts from *P. hydropiperoides* manifests a high insecticide power affecting the nervous system and the cellular respiration of the insects (Lizarazo et al., 2008). Additio-

nally, this component could execute a deterrent feeding effect and, at the same time, affect biological functions of the insects like silk worms, aphids, white fly larvae, thrips and some kinds of mosquitoes till even cause them death (Davidson, 1930; Ducrot, 2004).

According to the results of the insecticidal treatment, this could be explained because the dichloromethane extracts could present a higher concentration of components with insecticide activity, therefore implying that dichloromethane has a better performance in the extraction of the bioactive components, as it has been reported in previous studies (Lizarazo et al., 2008).

In addition, it can be observed from our data that the dichloromethane extract concentrations are far superior to the one of the commercial product. The bioactivity of the extracts implies the presence of active substances in its composition. These compounds might be purified and concentrated

aiming to obtain an insecticide with the same activity than the commercial product.

## CONCLUSIONS

Based on the results found it is possible to state that the extracts obtained from *Polygonum persicaria* present inhibitory feeding and insecticide activity over the *Nomophila indistinctalis*.

When comparing the control and the case in the antifeedant activity, there are only significant differences ( $p < 0.05$ ) after 90 minutes of exposition.

Among the prepared extracts, those obtained with dichloromethane present an insecticide activity similar to the one of the commercial product Neem in its maximum concentration proposed by the supplier. Although the difference in the concentration among them, the former's effects are evidence of the presence of active substances that could be used to produce an insecticide of similar activity of the commercial formula.

There are no differences in the extracts obtained with the dichloromethane solvent from the samples of *Polygonum persicaria* collected in Valparaíso or Curico.

Finally, to determine the differences in the insecticide activity of the different extracts (EtOH, MeOH, DCM) accurately, it is necessary to know the chemical composition of every extract, establishing its peculiarities and analyze the possible synergetic behavior that can arise to attribute the studied activities.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution	Quesada-Romero L	Fernández-Galleguillos C	Bergmann J	Bravo MA	Fuentes-Contreras E
Concepts or Ideas	X	X	X		X
Design	X	X	X		X
Definition of intellectual content	X	X	X		
Literature search	X	X			
Experimental studies	X				
Data acquisition	X				
Data analysis	X				
Statistical analysis	X			X	
Manuscript preparation	X	X		X	
Manuscript editing	X	X	X		
Manuscript review	X	X	X	X	X

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