ACTIVITY OF NONPOLAR EXTRACTS FROM *PICRASMA CRENATA* (SIMAROUBACEAE) AGAINST *MYZUS PERSICAE* (HEMIPTERA: APHIDIDADAE)

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ABSTRACT

The insecticidal activity of nonpolar extracts from *Picrasma crenata* (Vell.) Engl. was tested on *Myzus persicae* (Sulzer). Two trials were conducted, in both of them, the experimental unit was a leaf of strawberry (*Fragaria* x *ananassa*). In the first experiment, the extract solvents were petroleum ether (PE), dichloromethane (DC) and ethyl acetate (EA) at 3000 ppm and 6000 ppm (controls: methanol and water). The mortality rate was recorded at 6, 12, 24 and 48 hours. Data was analyzed by ANOVA and Tukey test ($p \leq 0,05$). At 48 hours, mortality was 75% for PE in each of the concentrations, 90% and 93% for DC and 100% for both EA concentrations. In the second experiment the dichloromethane extract was evaluated at 6000 ppm and 12000 ppm. Both concentrations produced 100% mortality. The ethyl acetate and dichloromethane extracts showed a significant toxic activity; however, the first demonstrated more effectiveness at lower concentrations. *www.relaquim.com*

Key words: *Picrasma crenata, Myzus persicae, Fragaria* × *ananassa,* insecticidal activity.

RESUMEN

La actividad insecticida de extractos no polares de *Picrasma crenata* (Vell.) Engl. fue evaluada sobre *Myzus persicae* Sulter. Se llevaron a cabo dos ensayos, en ambos la unidad experimental fue una hoja de fresa (*Fragaria* x *ananassa*). En el primer experimento, los solventes extractantes fueron éter de petroleo (EP), diclorometano (DC) y acetato de etilo (AE) a 3000 ppm y 6000 ppm (controles: metanol y agua). La mortalidad se midió a las 6, 12, 24 y 48 horas. Los datos fueron analizados por ANOVA y prueba de Tukey ($p \leq 0,05$). A las 48 h la mortalidad fue de 75 % para EP,

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para cada una de las concentraciones, 90 y 93 % para el DC y 100% para ambas concentraciones de AE. En el segundo experimento el extracto de diclorometano fue evaluado a 6000 ppm y 12000 ppm. Ambas concentraciones aniquilaron al 100% la población de afidos. Los extractos de acetato de etilo y diclorometano mostraron actividad tóxica significativa sin embargo, el primero demostró mayor efectividad a bajas concentraciones. *www.relaquim.com*

Palabras clave: *Picrasma crenata, Myzus persicae, Fragaria* × *ananassa* , actividad insecticida.

INTRODUCTION

Among the various strategies available to control pests, those that include the use of synthetic insecticides are the most commonly used. However, its use in unsuitable doses may cause resistance, pest resurgence, phytotoxicity and environmental pollution. Since they accumulate in eatable plants, insecticides can be toxic to humans and other mammals and also may eliminate beneficial insects (Alonso *et al.*, 1996). To develop an economically and ecologically sustainable agriculture in the medium- and long-term, it is imperative to seek new strategies of pest management (Silva *et al.*, 2002).

Some plant chemicals, such as secondary metabolites, are effective in controlling populations of insect pests. In general, they do not produce toxic residues that may affect wildlife and humans (Davidson, 1992).

The action of secondary metabolites on different species of insects varies, and it depends on their ability to metabolize the active substances and turn them into a harmless product (Hedin, 1982). Vascular plants have evolved over 400 million years, developing mechanisms to protect themselves against insect attacks, as repellents the insecticidal actions (Silva *et al.*, 2002). More than 60 years of research on the phenomenon of resistance have generated a great amount of valuable information to understand how arthropods, especially insects and mites, have developed a remarkable ability to live and reproduce in highly contaminated environments with pesticides (Bisset, 2002). So the search of new chemical structures derived from vascular plants is important for the development of emerging lines of research, with low costs and low environmental impact with the greatest potential for agricultural pest control.

In Argentina, some native Simaroubaceae are used for biological control. *Picrasma crenata* (Vell.) Engl. (syn. *Aeschrion crenata* Vell.) grows in the Northeast Argentina, Misiones (Vitagliano and Comin, 1971). This species is related to other species of Simaroubaceae, such as *P. excelsa* (SW) Planch., *P. quassioides* Benn, *Picramnia pentendra* SW, *Simarouba glauca* DC and *S. tulae* Urban. (Woodbury *et al.*, 1974).

From secondary stems of *P. crenata* several quassinoids have been isolated: quassine, neoquassine, isoparaine, paraine, 12-norquassine and picrasmine (Vitagliano and Comin, 1971) and two alkaloids: crenatine and crenatidine (Sánchez and Comin, 1971; Yoshikawa *et al.*, 1995); some of these showed antifungal activity (Ishii *et al.*, 1991). Quassine, neoquassine and picrasmine are used as natural insecticides (Mambelli *et al.*, 1994). These compounds have insecticidal action on several species of Hemiptera, Lepidoptera and Coleoptera (Stoll, 1989).

Quassinoids can be classified into different groups according to their basic skeleton: C18, C19, C20, C22 and C25; most of which occur with quassine containing C20 basic skeleton. These substances are

derived from the series of triterpenes eufol/tirucalol, most of which contain highly oxygenated lactones in their basic skeleton; more than one double connection rarely occurs (Guo *et al.*, 2005; Beserra Almeida *et al.*, 2007).

Quassine is characterized by oxygenated functional groups in their skeletons, except for C-5, C-9 and the methyl groups at C-4 and C-10 positions (Guo *et al.*, 2005; Beserra-Almeida *et al.*, 2007). Polonsky *et al.* (1989) tested the antifeedant activity of a series of quassine on *M. persicae*: isobruceine A, isobruceine B, bruceine B and C, and quassine glaucarubinone. All compounds were first tested at a concentration of 0,1 % a.i., painted on the leaf surface at ca. 0,01 ml/cm^2 (250 ppm w/w) of leave. Compounds active at this concentration were then tested at successively lower doses (0,05; 0,01; 0,005%) until anti-feedant activity ceased. We observed that feeding decreased with concentrations of 0.05 or lower, whereas isobruceine A was effective at 0,01%.

Regarding the insecticidal activity of quassine, it was observed that it has an impact on the hop aphid (*Phorodon humuli* [Schrank]) (Rosella *et al.*, 1991), feeding deterrence effects on the worm chewing (*Spodoptera eridania* Stoll.) (Lepidoptera: Noctuidae) (Guo *et al.*, 2005) and Mexican bean beetle (*Epilachna varivestis* Mulsant) (Coleoptera: Chrysomelidae) (Latif *et al.*, 2000).

Moreover, lethal effects of extracts of *Quassia* sp. Aff. bidwillii (Simaroubaceae), at concentrations of 10.000 ppm, on the common red mite, *Tetranychus urticae* Koch (Acarina: Tetranychidae) and the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) were found (Latif *et al.*, 2000).

Biological activity with *Q. amara* methanol extracts at 2% and 5% on *Tribolium castaneum* Herbst was observed. Repellence values were 89% to 93%, respectively, as measured by an olfactometer. Mortality was 10% to 20% for each of the concentrations (Dal Bello and Padin, 2006).

In the Department of Agricultural Zoology, School of Agronomy, University of Buenos Aires, promising tests have been developed with extracts of *P. crenata* with solvents of increasing polarity, to determine the insecticidal action and the optimum lethal concentration. These studies were conducted on stored grain pests mainly from the order Coleoptera (Rodríguez *et al.*, 2004; and Rodríguez *et al.*, 2008). An experiment was made on *Oryzaephilus surinamensis* adults. Different concentrations of ether of petroleum, dichloromethane and etil acetate extracts were used (0,15; 0,20; 0,25; 0,30; 0,35 and 0,40 g/ml). The mortality was examined from 0,5 h to 72 h, every 6 hours. The ethyl acetate extracts were the most effective. The 0,25 g/ml concentration produced a high mortality (90 % to 6 h),

The objective of this study was to evaluate the effect of nonpolar extracts from *P. crenata* on green peach aphid (*Myzus persicae,* Sulzer).

EXPERIMENT

The extracts were obtained from the Pharmacobotanic Laboratory of the School of Pharmacy and Biochemistry, University of Buenos Aires. A piece of wood from *P. crenata* (Vell) Engl. (Simaroubaceae) was used. Three solvents were used to obtain the extracts: ether of petroleum, dichloromethane and ethyl acetate.

Extracts collection

Pieces of wood (100 g) were ground with a rotary blade mill. It was placed in a 1000 mL Erlenmeyer flask and added 500 mL of absolute methanol (100%). It was left to soak for 48 hours in standard conditions, stirred regularly and then filtered. The methanol solution was obtained, as well as plant material.

A methanol-water solution (80%) was added to the plant material. Then, the previous sequence was replicated. Finally, the plant material was added methanol-water at 50% and the sequence was repeated. It was filtered and the plant material was thrown away and the three solutions were put together. Afterwards, the organic solvent was removed at low pressure with a rotary evaporator. An aqueous solution was obtained; it was partitioned with solvents of increasing polarity: petroleum ether, dichloromethane and ethyl acetate. The solvent was removed from each fraction and the yield was determined (g extract/g of wood bitter).

Quality control

The quassinoids of the extracts were determined through a high revolution liquid chromatography (HRL); column: Lic chro cart 250-4RP-18 (5 µ); solvent: ortophosphoric acid 0,02; molar: methanol: acetonitrile (50:35:15); flux: 0,5 ml/min.; detection: 255 nm; simple: 10 µl; standard solution: 25 mg/50 ml in mobile phase (0,5 mg/ml); control solution: 140 mg/25 ml in mobile phase (5,6 mg/ml); quassine: (RT: 15 min.); neoquassine (RT: 20 min.) (Figure 1).

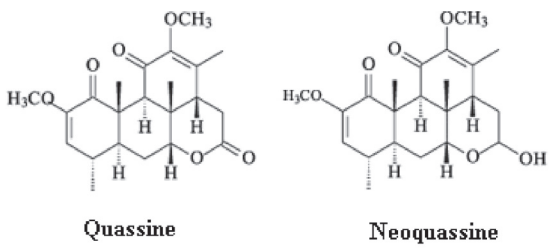

Figure 1. Chemical structure of Quassine and Neoquassine

Insects assayed

Apterous adults of the aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), were collected from strawberry (*Fragaria vesca* L. and *Fragaria x ananassa* Duch.) and multiplied in standard conditions (25 ± 1°C, 60± 5% RH and photoperiod 16:8 L: D). In addition, aphids were reared on strawberry plants in the laboratory. After several generations in this host, apterous viviparae of *Myzus persicae* (Sulz) (Hemiptera, Aphididae) were isolated for testing. The methodology consisted in leaving adult females of the same age over the surface of the plant; once the nymphs were born, adults were removed. When these nymphs became adults, they were used in the test.

Bioassays

Two trials were carried out. In the first experiment, we evaluated the effect of petroleum ether, dichloromethane and ethyl acetate extracts from *P. crenata* on adults of *Myzus persicae* Sulzer. We worked with 4-leaf seedlings from commercial suppliers at laboratory conditions (25 ± 1°C, 60 ± 5% RH and photoperiod 16:8 L: D). The experimental unit consisted of a box of 10 x 5 x 3 cm whose bottom was uniformly covered with wet cotton and filter paper. A strawberry leaf was placed on this filter paper. The leaves were sprayed with extracts of *P. crenata*, in methanol solution and by a De Vilbiss blister (20 lbs/inc.^{2}). Treatments were as follows: T_0: distilled water; T_1: methanol; T_2: 3000 ppm (ether of petroleum); T_3: 6000 ppm (ether of petroleum); T_4: 3000 ppm (dichloromethane); T_5: 6000 ppm (dichloromethane); T_6: 3000 ppm (ethyl acetate); T_7: 6000 ppm (ethyl acetate). The mortality rate was recorded at 6, 12, 24 and 48 hours. ANOVA (treatment x time) and Tukey test were used ($p \leq 0.05$). The assumptions of homocedacy and normality were verified by means of Levene and Wilk-Shapiro tests; data was transformed when necessary to fulfil both criteria (Brower, 1997; Zar, 1999).

In the second experiment and given the results obtained in the first experiment, increasing doses of dichloromethane extract of *P. crenata* (12,000 ppm), we carried out

the same procedure. We worked at laboratory conditions: (25 ± 1°C, 60 ± 5% RH and photoperiod 16:8 L: D). Treatments were: T_0: distilled water; T_1: methanol; T_2: 6000 ppm (dichloromethane); T_3: 12000 ppm (dichloromethane). The experimental unit was the same too. The mortality rate was recorded at 6, 12, 24 and 48 hours. Once again, ANOVA (treatment x time) and Tukey test were used ($p \leq 0.05$). For both trials, we used a completely randomized design; five replications of each treatment were carried out and 10 apterous adults of the same cohort of *M. persicae* and one day age in this state, were added to each replicate.

RESULTS AND DISCUSSION

No previous work has been reported on *P. crenata*. Some authors have been worked on others Simaroubaceae as *Quassia* sp. aff. bidwillii. Latif *et al.*, 2000 relationship with the mortality effect of quasinoid chapparinone on *M. persicae* and *Tetranychus urticae*. In futher tests, chapparinone showed the following activity (LC50 = Lethal Concentraton for 50% death): *M. persicae* LC50 = 14,9 ppm, *T. urticae* LC50 = 47,0 ppm. Chapparinone has previously been reported to show activity against the tobacco budworm (*Heliothis virescens* LC50 = 30 ppm), the best performance on *M. persicae* was observed.

There is coincidence with Polonsky *et al.* (1989) who tested the antifeedant activity of a series of quassine (isobrucein B, brucein B and C, glaucarubinone and quassin) on *M. persicae*. Only quassin showed no phytotoxic effects and is therefore the most promising compound for further development.

The effect of different extracts and different concentrations was analyzed over time. In the first experiment, an increase of mortality of *M. persicae* through time for all extracts was observed (Fig. 1). The best response was obtained with the ethyl acetate extract, in accordance with Rodríguez *et al.*, 2008) its high effectiveness was tested on *Sitophilus oryzae* (L.), two solvents were used to obtain the extracts: acetone and ethyl acetate. Different concentrations of extracts were used (0,15, 0,20; 0,25; 0,30; 0,35 and 0,40 g/ml), mortality was examined from 0,5 h to 72 h, every 6 hours. The treatments 0,30g/ml, 0,35g/ml and 0,40g/ml showed a100% efficiency after 6 hours to ethyl acetate extracts. Regarding the acetone extract, all the concentrations ranged between 80 and 100% of efficiency after 6 hours, reaching 100% after 18 hours.

In this assay, the dichloromethane extracts showed an efficiency of 90% and 95%, respectively, after 48 h. Although after 48 h concentrations of the extracts of dichloromethane and ethyl acetate were equally effective, a lower concentration of ethyl acetate (3000 ppm) showed better performance for the control of the pest, characterized in the first hours of the test. No statistically significant differences between the ethyl acetate and dichloromethane extracts were obtained ($p < 0.05$)

Petroleum ether only achieved an efficiency of 65%, there is coincidence with Iglesias *et al.* (2009) who obtained a tumbling effect of 66% on adults of *Ceratitis capitata* (Wied) at a concentration of 5000 ppm.

The methanol control produced a certain degree of mortality; it does not differ statistically from the water control (Figure 2).

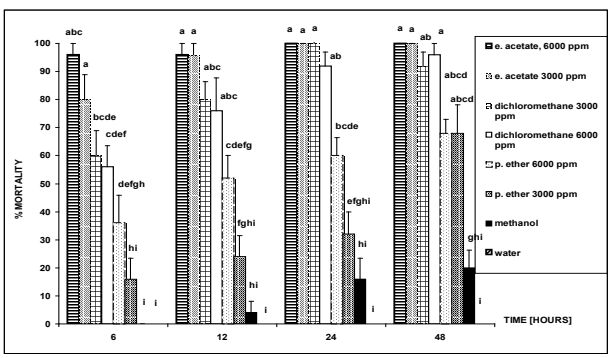

Figure 2. *Myzus persicae* Sulzer mortality with ethyl. acetate, dichloromethane and petroleum ether extracts along time. Different letters indicate significant differences for Tukey test ($p \leq 0.05$). The bars represent the standard error

The results of this first experience indicate a slight superiority (5-10%) of dichloromethane extract in controlling *M. persicae* with a concentration of 3000 ppm over the same extract with a concentration of 6000 ppm up to 48 hours, in which the results are reversed. However, no statistically significant differences were obtained ($p< 0.05$). For this reason, a second experiment was carried out, it repeated the concentration of 6000 ppm and a doubled dose was tested. This is possible since the quasinoids have demonstrated not be toxic for humans. Many molecules display a wide range of inhibitory effects, including anti-inflammatory, anti-viral, anti-malarial and anti-proliferative effects on various tumor cell types (Fiaschetti *et al.*, 2011).

The results indicate a 100% mortality at 12 hours with both concentrations (6000 ppm and 12000 ppm), the differences were not statistically different from the lowest concentration. Preliminary work resulted in a mortality rate no greater than 60% of *Oryzaephilus surinamensis* in the adult stage when extraction solvents were those of the non-polar (Rodríguez, *et al.*, 2005). It suggests the existence of a differential effect, depending on the host. In this trial, dichloromethane (6000 ppm) showed higher efficiency to 6 and 12 hours, 80% and 100%, respectively, than in the first experiment. After 24 hours the effectiveness was high in both trials (Figure 3).

This suggests the existence of effects that could prevail over those of the extracts. This requires the continuity in research. Therefore, one should consider the economic costs would imply an increase in dose. The toxicity of the extract at higher concentrations would not be a limitation for the reasons stated.

The ethyl acetate with a concentration of 3000 ppm produced 80% mortality after 6 hours and 100% after 12 hours. Therefore, the ethyl acetate extract is the most effective for its high efficiency.

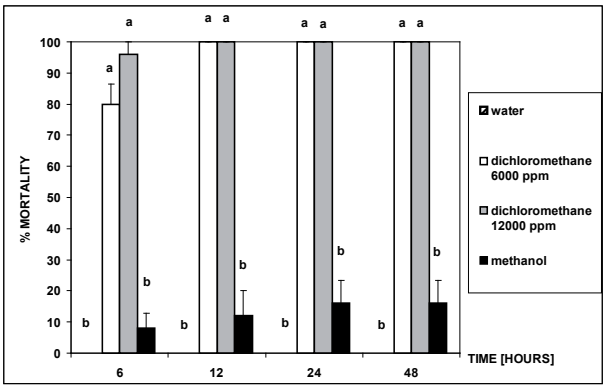

Figure 3. *Myzus persicae* Sulzer mortality with dichloromethane extracts along time. Different letters indicate significant differences for Tukey test ($p \leq 0.05$). The bars represent the standard error.

CONCLUSIONS

Recently, an increase in insect pest resistance to conventional insecticides has been observed. This can be attributed to decades of indiscriminate, continuous and repeated use. Therefore, an alternative can be a bio-rational management together with or without a small amount of conventional pesticides. Several plants with biocidal properties such as *P.crenata* can be a good choice to address key pests. This experience has demonstrated the effectiveness of non-polar extracts of *P. crenata* plant species on *M. persicae*. *P. crenata* grows over a wide area of northeastern Argentina and it is not threatened in this ecosystem; this non-timber resource is used rationally, complying with the Forestry Law.

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