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# Exposition of Pecan Black Aphid (*Melanocallis caryaefoliae*) to Creseote Bush (*Larrea tridentata*) Extracts

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## Abstract

**Creseote bush-based (*Larrea tridentata* Cov.) botanical insecticides have not been evaluated on pecan black aphid (*Melanocallis caryaefoliae* D.), the purpose of this research was to test the insecticide and/or repellent effect of the creseote-bush raw extracts on this insect. Ethyl acetate, methanol and water as solvents, the stem and leaf organs were tested at concentrations of 0.5%, 1%, and 2%, as well as their witnesses and the interactions of each. Extract application was made by immersion during 10 s and the incubation was made in a wet chamber, making observations at 24, 48 and 72 hours after the treatment was applied. Mortality was evaluated by touching the aphid and seeing it did not move, while for repellence the insects found outside of the leaf were taken into account. A higher mortality effect was observed at a 1% concentration for the three solvents on the leaf extracts, being the methanol one the most efficient, on the other hand the stem extracts had the same behavior, presenting the higher mortality in the ethyl acetate extracts at 0.5% and 2% both at 72 hours. On the other hand the higher repellent effect presented at 24 hours in stem extracts, with a variation in the leaf extracts.**

## Keywords

**Botanical Insecticides, Repellent Effect**

## 1. Introduction

Vast use of insecticides has polluted the fields and water bodies, also plague insects have developed resistance

to several chemical products and what once were secondary plagues have now become major plagues.

Creseote bush (*Larrea tridentata* Cov.) is one of the most prolific native plants of the North American desert, it belongs to the Zygopylaceae family, with many potential applications [1], such as the medicine which is used in the treatment of many different diseases, mentioned by [1] [2] and [3]. Creseote bush's active compound is Nordihydroguaiaretic acid (NDGA) which can block cellular respiration and antioxidant activity at a cellular level, inhibits lipoxygenase, ornithine decarboxylase (on mice) and collagen induction [4]. It has also been found that a NDGA derivate inhibited the human papilloma virus and herpes simplex [5].

In agriculture the creseote bush resins have showed fungicide activity against *Fusarium oxysporum*, *Botritis cinérea* reported by [1], also [6] reported there was an inhibition to the growth of *Aspergillus flavus*, *A. parasiticus Pythium spp*. fungi of 92% and 86% respectively, among others.

Insecticide activity has also been observed against bean weevil, *Acanthoscelides obtectus* (Coleoptera: Bruchidae), as well as against the larger grain borer, *Prostephanus truncaturs* (Coleoptera: Bostrichidae) [7]; repellence was observed in the bean weevil *Zabrotes subfasciatus* not being attracted to the stored pinto bean seeds previously treated with *L. tridentata* leaf and flower powder [6]. In 2006 Viglianco *et al.* [8] reported that the raw extracts of *Larrea divaricata* C. leaves and stems extracted with ethanol, hexane and chloroform produced a repellent and antifeedant effect on *Sitophilus oryzae* L.

Tarango, (2006) [9], informed that the pecan (*Carya illinoensis* K.) is the most important deciduous fruit in Northern Mexico and one of the major plagues in this crop is the pecan black aphid (*Melanocallis caryaefoliae* D.). Fu *et al.* (2007) [10] point out that the general damage of the aphids is a reduction in the photosynthetic foliage surface through defoliation, leaf size, chlorophyll and light absorption reduction, decreasing the yield. In a survey made by Cottrell *et al.*, (2009) [11] they observed that the pecan black aphid tends to feed on foliage with a certain degree of chlorosis, and if there is not such, they induce it so the pecan responds increasing the amount of amino acids on the leaf, they also mention the aphids which remain on the leaf have more chances of surviving and increasing their population than those which move to try to find another feeding zone. Taking into account that a pecan black aphid may produce a dead area of 0.635 $cm^2$, a severe infestation can cause 8 - 10 spots per leaflet, ending with premature fall of compound leaves, with the potential for defoliating a whole farm [12]. For these reasons it is important to keep this insect controlled and one alternative is using botanic products which eliminate or repel the aphids, also with a minimum harmful effect on the predators which help to control this aphid.

In this survey are described the laboratory bioassays to determine the biological activity of the creseote bush's raw extracts, their insecticide and/or repellent effectiveness on the pecan black aphid and the $IC_{50}$, on exposure, as well as to determine the efficiency of each plant organ tested (stem and leaf) as well as the solvent used to extract the creseote bush's secondary metabolites. Having plenty of medicine applications, creseote bush's extracts may be a good alternative for controlling the pecan black aphid without affecting the environment, beneficial organisms or man.

## 2. Experimental Methods

### 2.1. Plant Material Collection

The plant material collection was made in May 2008, in the municipality of Aldama in the state of Chihuahua. Creseote bush samples were taken randomly. In the laboratory the plant material was washed with water jet, the organs were separated (leaf and stem), they were left to dry at room temperature, then they were set in paper bags to dry on a stove for 24 hours at a temperature under 50˚C, once they were dried they were crushed until a fine powder was obtained.

### 2.2. Extract Preparation

For the preparation of the organic extracts were used the solvents Ethyl Acetate and Methanol. Each of the plant parts was macerated in the organic solvents at room temperature, to separate the plant material extract they were filtered using Whatman Grade 1 filter paper. The aqueous extract was macerated for 24 hours, it was filtered in the same manner, and with the filtered material a stock solution was obtained. The organic extracts were obtained by separation from each solvent, using a rotary evaporator at 70 rpm at a temperature lower than the boiling point for each solvent.

Doses at 0 (witness), 0.5%, 1.0% and 2.0% were evaluated, besides of the solvents, their interactions and distilled water. The survey had 4 repetitions by treatment and 10 individuals for repetition; to obtain the concentrations of each organic extracts the methodology of Lira-Saldivar *et al.* 2003 [6] was used, where a sodium hydroxide solution at 3% was added to re-dissolve them in water, later they were diluted until they set at 0.3% sodium hydroxide (to avoid mortality and fitotoxicity). The aqueous extract concentrations were made by weighting the previously calculated amount for each (weight-volume ratio), next it was dissolved in distilled water calibrated at 50 ml in a flask.

### 2.3. Bioassay of the Bio-Insecticide Activity

The pecan black aphid was collected in pecan farms (Aldama, Chih. and Hermosillo, Son.) with infested leaves, they were transported to the laboratory in plastic bags inside of ice buckets at a temperature lower than environmental temperature to decrease the activity; the bioassay was performed following the immersion technique described by Lagunes (1994) [13], with a wet chamber where the petri dishes were identified and each was provided with filter paper with 1.5 ml of water to keep humidity. Labeled glass flasks were used for each concentration, the aphids were set on the dishes, then a piece of leaf was taken with the aphids and immersed in the treatment for 10 s, the treatments were incubated at a maximum temperature of 25˚C and minimum 14˚C, and photoperiod of 16:8 (L:O), the readings were made every 24, 48 and 72 hours. To measure the aphids' mortality it is considered the aphids that move after teasing them with an entomological needle. To measure the repellence effect the insects that abandon the leaf or that stay on it are considered, taking into account that any aqueous or gaseous phase chemical substance causing that the insect leaves its food source is considered repellence [14].

### 2.4. Statistical Analysis

The experimental design was completely at random with a factorial arrangement. The statistical package SAS 9.1 was used to obtain the ANAVA. The results obtained were subjected to median comparison through Tukey ($\alpha = 0.05$), to eliminate the solvent effect the orthogonal contrast test was used. The variables evaluated were the repellent effect and insect mortality.

## 3. Results and Discussion

### 3.1. Effect of Extracts on the Number of Dead Aphids

The different concentrations of aqueous extracts observed at 24 hours of exposition did not affect the aphids, on the other hand the methanolic extract did affect the aphids causing their death at the different concentrations and organs tested ($\alpha = 0.05$) (**Figure 1** and **Figure 2**), when testing the ethyl acetate extract which has a lesser polarity, it was observed that only the stem shows a significant difference at the different concentrations.

At 48 hours only the stem methanol extract keeps the effect on the aphids' death with the concentrations tested, while at 72 hours a significant difference is observed only with the leaf aqueous extract at 1% with results of up to 80% of dead insects.

In **Figure 1**, it is observed the positive trend of the different leaf extracts' mortality effect and their concentrations through time; in the 3 types of extracts, the one with a greater effect on aphids was the 1% dose in the three solvents tested, showing up to 80% and 92% mortality in the aqueous and methanol extracts, accordingly. At the same time in **Figure 2** the effects of the stem extracts are observed where the higher mortality effect produced by extracts was 0.5% for ethyl acetate, 1% for methanolic and 0.5% for aqueous extract, in all extracts the greater effect is given at 72 hours after the treatment was applied.

The concentrations of the several leaf and stem extracts were compared statistically against the witnesses used and it was observed that the mortality effect is due to the secondary metabolites extracted from the creseote bush and present in the tested extracts, not from the substances used to extract them.

### 3.2. Effect of Extracts on the Number of Repelled Aphids

In respect to the repellent effect of the different extracts and their concentrations a significant difference was found only in the methanol leaf extracts at concentrations of 1% and 2% 72 hours after the treatments were applied.

It was observed that in general the greater repellent effect is reached 24 hours after the treatment is applied and it decreases afterwards; in the ethyl acetate, methanol and aqueous leaf extracts the greater repellence was observed at 0.5% concentration at 24 hours with a 45%, 65% and 35% of aphids set outside of the leaf (**Figure 3**).

In **Figure 4** the results of the stem extract repellent effect can be observed where there was also a decrease of such effect through time, showing the best results at 24 hours, where the 1% concentration produced a 50% and 35% repellence with the ethyl acetate and aqueous extracts respectively, while for methanol extracts the 0.5% concentration provoked that 37.5% of the insects were set outside of the leaf.

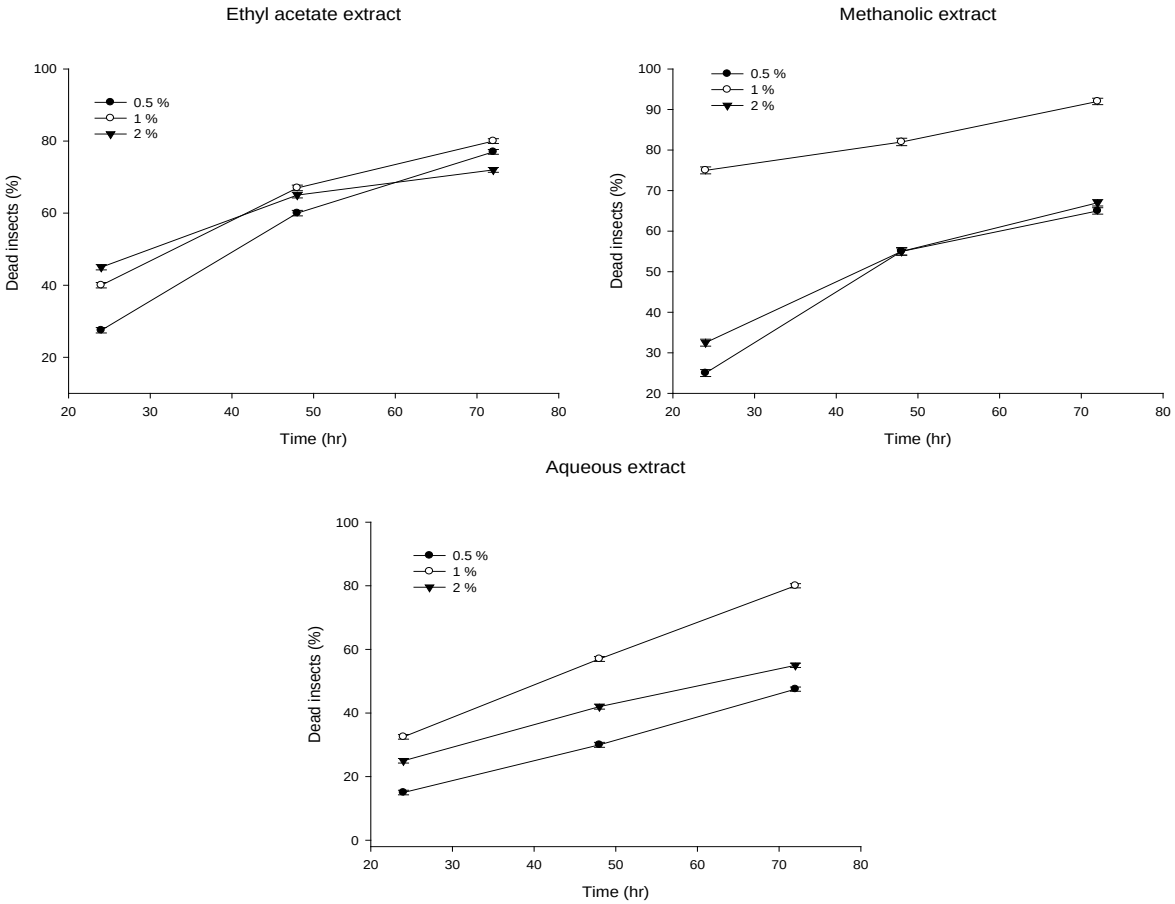
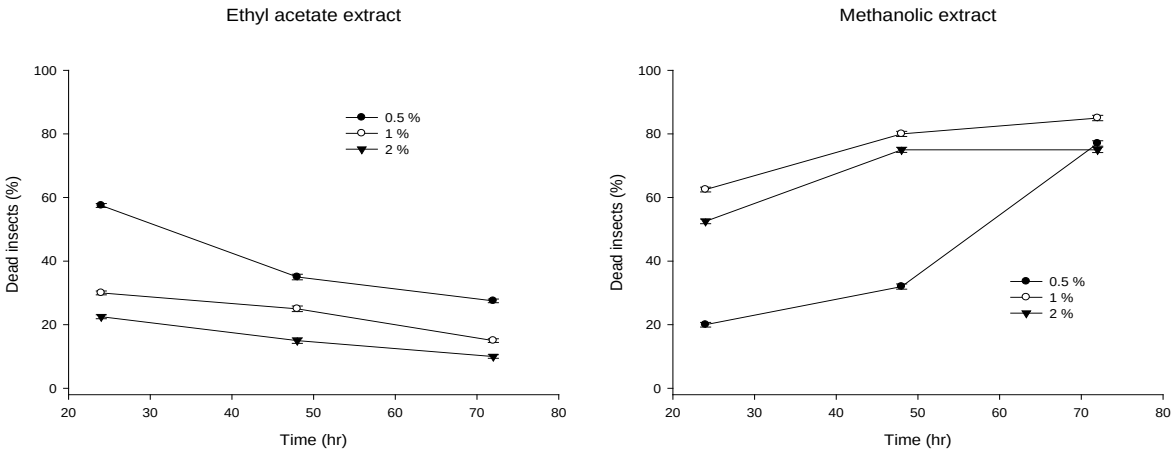



**Figure 1.** Effect of *Larrea tridentata* leaf extracts over the aphid mortality percentage through time.



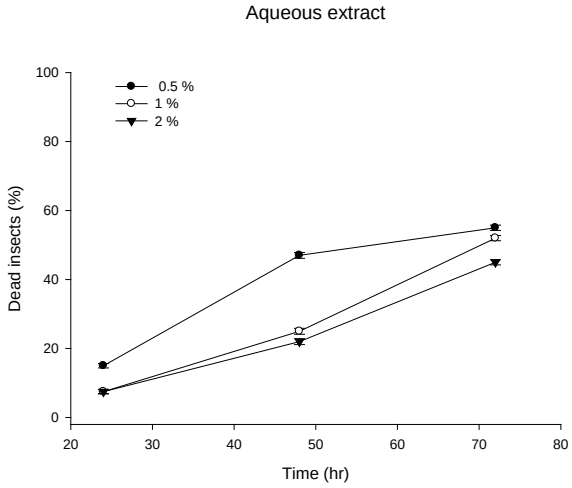



**Figure 2.** Effect of *Larrea tridentata* stem extracts over the dead aphid percentage through time.

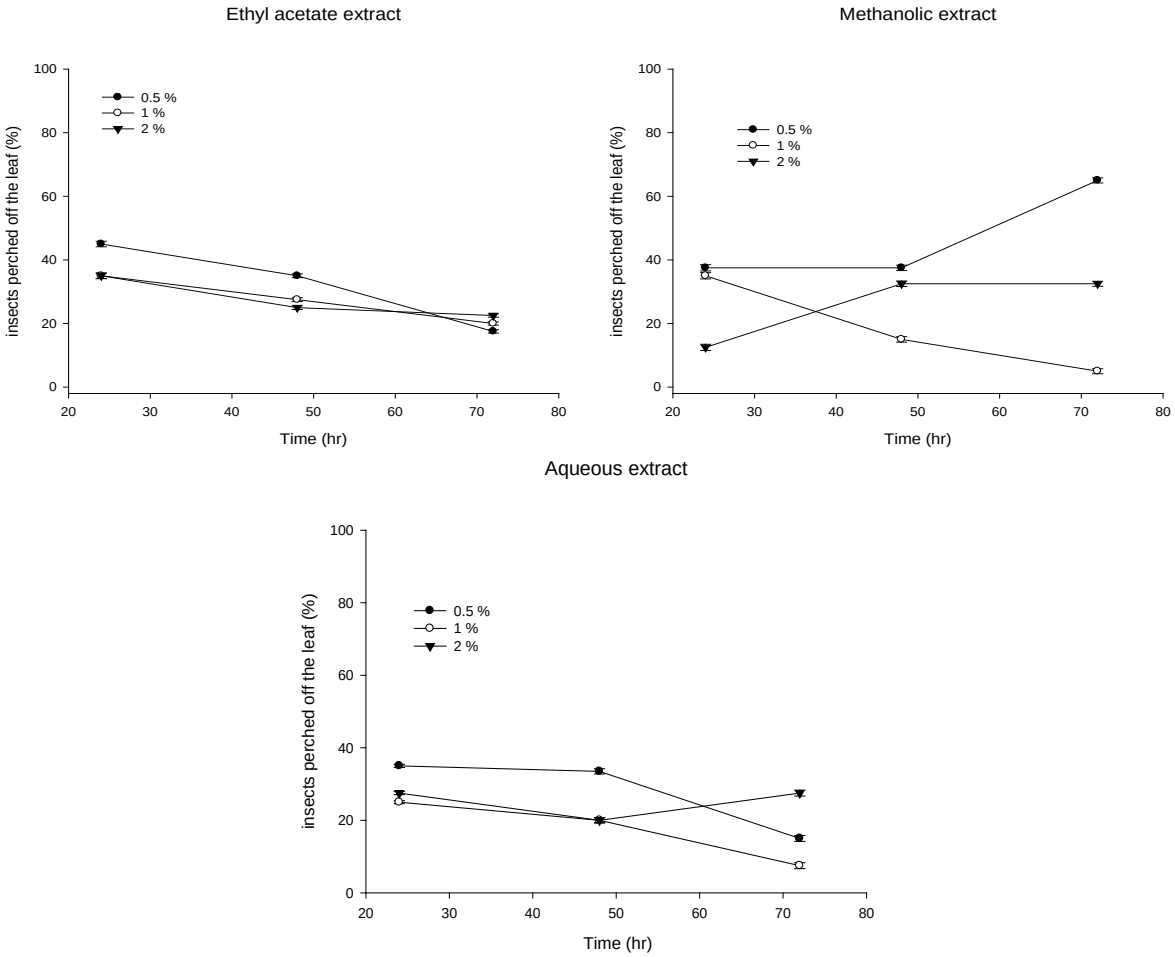



**Figure 3.** Effect of the *Larrea tridentata* leaf extracts on the number of aphids set outside of the leaf through time.

The extracts were compared to their corresponding witnesses and it was observed that the repellent effect was caused by metabolites extracteds from the creseote bush's stems or leaves and not by the solvents used, observing a trend different to the mortality effect.

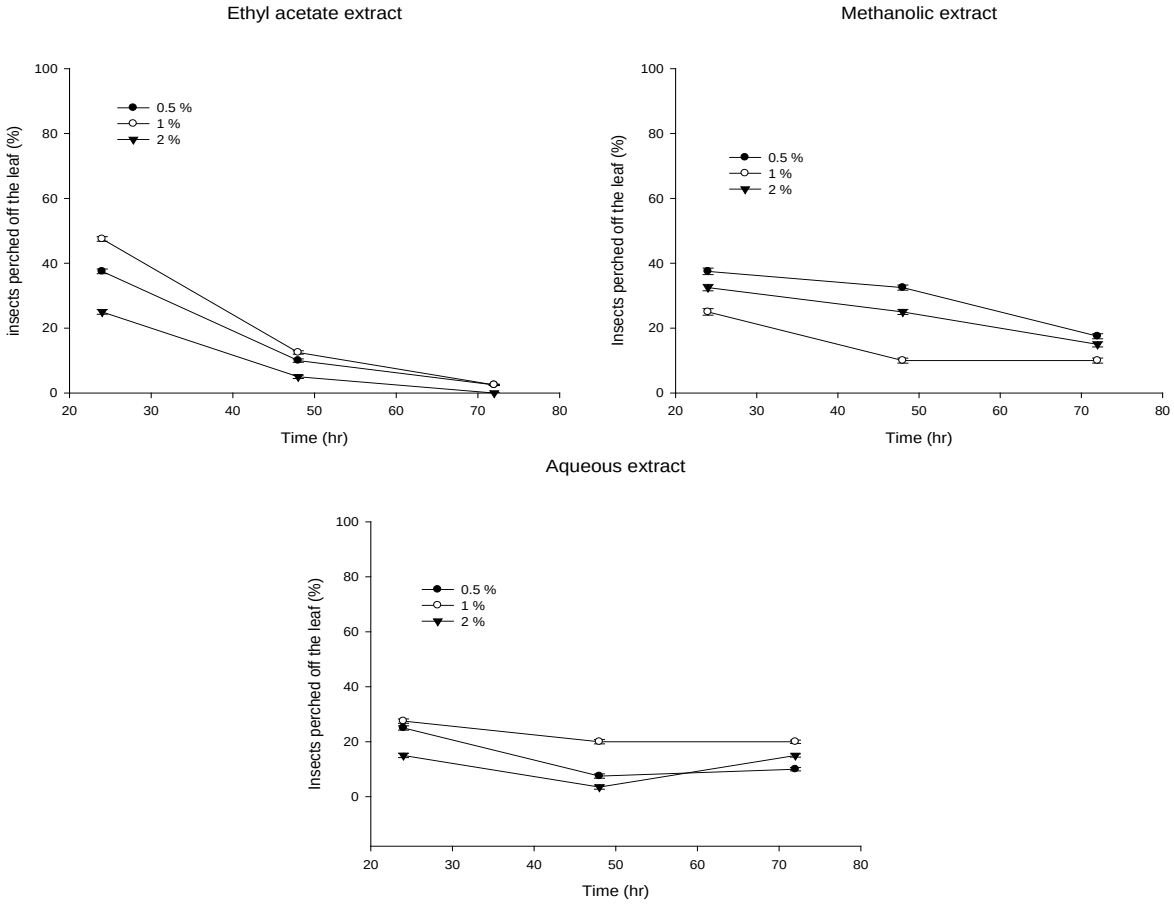



**Figure 4.** Effect of the *Larrea tridentata* stem extracts on the number of aphids set outside of the leaf through time.

## 4. Conclusion

With the results obtained in the laboratory it is concluded that the extracts of creseote bush extracted with ethyl acetate, methanol and water have the potential to be used as insecticides against the pecan black aphid, so it is recommended to evaluate each of the extracts and witnesses on the field at the different concentrations.

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