IN VITRO NEMATOCIDAL ACTIVITY OF *Psidium guajava* AND *Rossmarinum officinalis* AGAINST *Haemonchus contortus*

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Introduction. In Mexico, a number of plant species have been used as a traditional ethnobotanical medicine to cure human diseases; these plants have used for the treatment of diarrhea, dysentery, gastroenteritis and indigestion.[1] Anthelmintic resistance is dramatically increasing; therefore, novel methods of control need to be explored. *Haemonchus contortus* is a very pathogenic parasite affecting ovine industry around the world.[2] In this work was evaluated the *in vitro* anthelmintic activity of *Psidium guajava* and *Rossmarinum officinalis* extract against the fourth larval stage of *H. contortus*.

Methods. Lefts of guayaba (*P guajava)* were macerated with methano, while romero (*R officinalis)* were macerated with dicholorometane and methanol. All of them were achieved to obtain the liquid with the active chemical compounds. Solvent evaporation was achieved with a rotaevaporator.

Larval infectives of *H. Contortus* were obtained from fecal cultures prepared with feces from an *H. contortus*-infected sheep that had been experimentally infected in CENID-PAVET-INIFAP.

The bioassay was run in 24-well cell culture plates. Methanolic extract of romero was dissolved in Tween 20, 1% (v/v), dichlorometane extract of romero (*R officinalis*) was dissolved in Tween 80, 1% (v/v), and guayaba methanolic extract (*P guajava)* were dissolved in Ethanol 1% (v/v), to obtain 20 mg/mL. In each well of the cell plate, 80 µL of the corresponding extract was deposited. A larval suspension (20 µL) containing 100 *H. contortus* L_4 was deposited in the corresponding well. Three wells of the plates were considered as three replicates per treatment, including the proper controls to record the natural mortality from causes other than the plant extracts. Plates were incubated at room temperature (10-18 °C) for 69 h. Aliquots were taken at 21, 44 and 69 h of incubation. Then 5 µL drop aliquots were taken from each well and deposited on a slide for quantification under the microscope (4x magnification). Evaluation criteria were based on the means of live and dead larvae in the different treatments. Results were expressed as the percentage larval mortality. The mortality in the plant extract treatments was adjusted to take into consideration the natural mortality data from the negative control. The same numbers of wells containing larvae in ivermectin solution was used as the positive controls.[3]

Statistical Analysis. ANOVA test was used to compare the means of the different treatments followed by the Tukey test as a complementary method to determine which treatment was statistically different among the others.[4]

Results. The extracts showed moderates values of lethal activity against *H contortus* L_4. The lethal activity of the extracts is showed in figure 1. Highest values corresponded to the methanolic extract of *P guajava* and it was statistically different to the others. *R officinalis* extracts were not statistically different each other. It indicated that the slowly nematocidal activity of romero could be due to presence of several compounds.

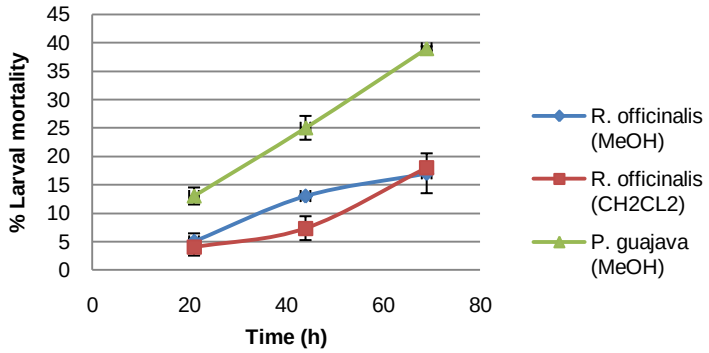

Fig.1 *In vitro* nematocidal effectiveness of *R. officinalis* and *P. guajava* extracts against *Haemonchus Contortus* forth larval stage.

Conclusions. This is the first time that *P. guajava* y *R. officinalis* extracts were evaluated *in vitro* for potential anti-nematodic effect against larvae of *Haemonchus contortus*. This nematocidal effect could be due to polar compounds like flavonoids, organic acids o glycosides.[1,6]

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