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Ethanolic extract of *Cedrela odorata* and *Delonix regia* for the control of *Anthonomus eugenii*

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Abstract
In recent decades, studies of plant-based products in their chemical part have been intensified, with an emphasis on secondary metabolites, which are involved in biological pest control and in some cases by activating defense processes in the providing preventive protection. This study evaluated the bioactivity of cedar (*Cedrela odorata*) and flamboyant (*Delonix regia*) extracts on adults of pepper weevil (*Anthonomus eugenii*). Mortality and repellency bioassays were performed in 150 ml volume bottles with various concentrations of extracts. The bioassays were completely random, with four repetitions for each treatment. In the assessed concentrations of *Cedrela odorata*, the mortality rate ranged from 20 to 30% of exposed insects. Concentrations of *Delonix regi*a extract have mortality effect on *Anthonomus eugenii* from 76% to 85% on individuals exposed in the test. With regard to the repellency effect, the extracts of both plants had low effectiveness.

Keywords: Bioassays, pest, agriculture, extracts

Introduction
Anthonomus eugenii Cano named as the pepper weevil is one of the most important pests in pepper crops [1], specializes in attacking crops of *Capsicum chinense* and *Capsicum annuum* [2]. The damage caused by the larvae is manifested in the small number of fruits, their early fall, premature maturation and the production of deformed fruits [1, 3].
Rodríguez-Leyva *et al*., (2012) [4] estimate that in Mexico they are lost from $70 million to $80 million annually from the attack by *A. eugenii*. Its control consists a chemical and cultural control, which keeps pest level infestations low [5], but in practice management falls on applications of insecticides like neonicotinoid (pyrethroids), were used widely by the producers, but created a new selection pressure on the populations of pest [6].
Pest control is commonly done with chemicals; however, irrational use of these agrochemicals, use of mixtures, use of ineffective products, application of persistent chemicals, inadequate equipment, increased frequency and dosage of applications leads to the elimination of insects the emergence of the resistance of major pests to pesticides, as well as water, air and soil pollution, accumulation of toxic waste and user poisoning [7]. This problem has driven the use of plant formulations with insecticide or insectistatic properties, which allow to manage pests, protect the crop and therefore obtain higher yield and quality in production without putting the health of man at risk and its environment [8]. Over the past two decades, studies of plant-based products have been intensified in their chemical part, with an emphasis on secondary metabolites, which are involved in biological control against pathogens or pests and in some cases activating processes of the plant and providing preventive protection [9].
In view of the mentioned before, this work was raised with the aim of evaluating the ethanol extracts of *C. odorata* and *D. regia* for an alternative to the control of for the control of *A. eugenii*.

Materials and methods
Plant material
Plant material was collected for the elaboration of extracts, Two kilograms of ripe fruits of *C. odorata* and two kilograms of leaves of *D. regia*. These were collected in the Botanical Garden of the Tizimin Institute of Technology. The collection was carried out in the early hours of the morning to preserve the turgor of the samples.

The leaves of *D. regia* were washed with plenty of distilled water to remove any residues, impurities or microorganisms present. Only the peel and seeds were removed to the fruits of *C. odorata*.

After washing the leaves of *D. regia*, these were spread over cardboard plates subjected to room temperature and protected in the shade for seven days and then introduced to the forced air stove at 60 ºC for 24 hours to ensure drying. This step was only performed with the sample of leaves of *D. regia*, in the case of the fruits of *C. odorata* were subjected to the forced air stove for 24 hours at 60 ºC.

Previously dried samples were processed in the Wiley type mill to obtain approximately one kilogram of sample for each species of plant collected. A 2 mm metal mesh sieve was used in the grinding of the samples, obtaining homogeneously sized particles. The result was stored in plastic bags and stored in a cool, dry place with their respective identification.

Ethanolic extraction

The samples were shaken to perform the ethanolic extraction as described below; two vessels of beakers of one liter capacity were taken and in each was poured 266 ml of reactive grade ethanol, in one glass five grams of the fruit of *C. odorata* ground and in the other ten grams of ground *D. regia* leaves. They were put in constant agitation at 110 r/min for a week at room temperature on a SBS-branded magnetic stirring heating plate. Over the course of the week, agitated sample decanting was performed every 48 hours, the liquid part of the bottom of each glass was separated, each residue was added the same amount of ethanol (266 ml) and was put back in agitation, the liquid part was stored in a 300 ml flat-bottomed flask and kept in refrigeration at 4 ºC, this procedure was performed three times for each stirred sample, at the end six decans were performed and six samples were obtained (three of *C. odorata* and three of *D. regia*) as a result of agitation.

Each sample resulting from the ethanolic preparation was distilled by thermo bath to obtain from each 20 ml, which were stored in an amber bottle and stored in refrigeration. This procedure was performed three times for each plant species. Each distillation lasted on average four and a half hours with the equipment available in the laboratory taking care that the water temperature of the thermo bath did not exceed 95 ºC. The result was cooled at 4 ºC.

The 20 ml resulting from each distillation were mixed according to the species of the plant for distillation, applying direct heat consisting of mixing in a beaker the total 60 ml of the samples previously distilled by thermo bath, this process was twice, once per species used, each extract was subjected to constant agitation at 110 r/min taking care that the temperature of the extract did not exceed 72 ºC in the distillation so as not to alter the secondary metabolites in the extract. The process lasted approximately 2 hours and 30 ml of each extract was obtained, which was used to perform the bioassays.

Collect the *Anthonomus eugenii*.

Infested fruit collections were collected with individuals from *A. eugenii* on plantations adjacent to the town of Yaxcheku, Tizimin, Yucatan, Mexico. Later they were subsequently taken to the parasitology laboratory of the Technological Institute of Tizimin to be processed, of which the first larvae and pupae were obtained that were protected in jars with pepper fruits.

Once the pupae fully developed and became adults, they were taken to the greenhouse with a habanero pepper crop to be released, in order to infest the plants and obtain adult individuals for bioassays. As planned, this activity took place at the beginning of the flowering of the plants. This process took approximately 45 days, between the months of November and December of 2016, giving the appropriate agronomic management to the plants to favor the development of *A. eugenii.*

In February 2017, the first catches were made in the greenhouse of the Institute, with these individuals mortality bioassays were carried out. Later in the month of May, collections of other adults of *A. eugenii* were carried out to perform the repellency bioassays.

Mortality bioassays

The procedure documented by Castillo *et al.* (2012) [10], was used in the bioassays with some modifications; four concentrations (0.5%, 1%, 2% and 5%) were prepared *(C. odorata* and *D. regia)* and a witness to perform the mortality test. Groups of 10 adults of *A. eugenii* were used, which were deposited in clear plastic bottles of 150 ml capacity at a temperature of 25 ± 2 ºC. The lids of the jars were perforated by placing a cloth mesh to prevent insects from coming out. The concentrations of the extracts were prepared according to the required presence, so that when preparing a 1% concentration, 0.10 ml of extract was used and 10 ml was graduated with distilled water used as a solvent.

To apply the treatments, filter paper (Whatman no. 1), trimmed in circles with a diameter of approximately 4 cm c/u, was used, which was completely impregnated with the extract corresponding to the treatment, then the paper circle impregnated with the extract, was introduced the bottle with the 10 individuals of *A. eugenii.*

The mortality effect of *C. odorata* and *D. regia* extracts was evaluated. Observations were made every 24 hours for four days to account for insect mortality for each treatment and repetition.

In mortality bioassays, before performing statistical analyses, the values (data) were adjusted according to the corrected Abbott formula [11]:

$$\% \text{ Mortality Corrected} = \left[\frac{\%mt - \%mta \quad X\ 100}{100 - \%mta} \right]$$

Where:

Mt = is mortality in treatment and

Mta = is mortality in witness treatment.

Repellency bioassays

For repellency bioassays, four concentrations (0.5%, 1%, 2% and 5%) were also evaluated by extract (*C. odorata* and *D. regia*) and a witness. In this test an election trial was proposed, using two 150 ml bottles, both had a hole located on one side of the bottle and connected with a transparent hose 1 cm in diameter and 5 cm long in such a way that a complex of two bottles attached to through a hose. In bottle No. 1 a circulate of filter paper (Whatman No. 1) with 4 cm diameter was placed, completely impregnated with the concentration of the extract to be evaluated, in the same bottle 10 adults of *A. eugenii* were released, the objective was to measure or observe the migration from one bottle to another of the insects exposed as a result of the possible repellent

effect of the extract evaluated. This was done under a temperature of 25 ± 2 ºC. The lids of the jars were perforated by placing a cloth mesh to prevent insects from escaping. The concentrations of the extracts were prepared according to the required presence, so that when preparing a concentration at 1%, 0.1 ml of extract was used and 10 ml was graduated with distilled water used as solvent.

The repellent effect of the extracts of *C. odorata* and *D. regia* was evaluated, observations were made over an eight-hour period, every hour the migration of the insects, from bottle one to bottle two, this was done for each treatment and every repetition.

Experimental design and statistical analysis

The bioassays it was done with a experimental desing totally random, with four repetitions for each treatment. The effect of the species from which the extract of *C. odorata* and *D. regia* was obtained on the corrected mortality and the cumulative repellency of *A. eugenii*, with a mixed-effect model where it is evaluated as factors the effect of the species and the concentration of the extracts with interaction, the time interval was 24, 48, 72 and 96 hours, and the replicas in this factor were a random effect. The concentration effect for each species was also evaluated using a mixed model separately, with the same condition as the most complex model. When one interaction was significant, we used a posteriori constrastes to test the differences between pairs of means for a given factor within each level of the other factor [12]. The concentration for each species, was evaluated with a mixed model using the function lme in the package nlme, and for the evaluation of the LC50 ($p<0.05$) was performed a Probit analysis using the function drc and dose in package MASS. Both analysis running in modules of the Software R [13, 14, 15, 16].

Results

For the corrected mortality bioassay, no significant effects were found among the species from which the extract was obtained, nor on the interaction between species and concentration (Table 1). However significant difference was found between the evaluated concentrations of *D. regia*. In the case of cumulative repellency we no found an effect of the extract type, concentration separately and in interaction were found. We find that *C. odorata* has a greater effect on repellency when applied at the highest concentrations for this study (Table 2).

Table 1: Effects of the type and concentration of the extracts as main factors evaluated with mixed models, on the time interval 24, 48, 72 and 96 hours and the replicas were nest in this factor as a random effect. P value (<0.05 in bold).

Corrected Mortality			
Source	**All extracts**	***C. odorata***	***D. regia***
Species	$F_{1,16}$=2.986	-	-
Concentration	$F_{1,16}$=0.133	$F_{1,16}$=0.204	$F_{1,16}$=0.0001
Species: Concentrations	$F_{1,16}$=0.073	-	-
Cumulative Repellency			
	All extracts	*C. odorata*	*D. regia*
Species	$F_{1,32}$=8.80	-	-
Concentration	$F_{1,32}$=150.65	$F_{1,32}$=4.00	$F_{1,32}$=0.802
Species: Concentrations	$F_{1,32}$=4.75	-	-

Table 2: Average repellency percentage for each type of extract, EE per concentration level (Conc).

Concentration	***C. odorata***	**EE**	***D. regia***	**EE**
0	3.13 [a]	1.69	0.63 [a]	0.55
0.5	7.81[b]	2.51	1.25 [a]	0.75
1	6.56[b]	2.49	1.56 [a]	0.82
2	8.13[b]	2.47	0.00[a]	0.00
5	7.50[b]	2.41	1.25[a]	0.75

Discussion

This experimental paper evaluated the extracts of the mature fruit of *C. odorata* and leaves of *D. regia* to see if they exhibit bioactivity as insecticides. To test the bioactivity of the extracts, *in vitro* tests were performed exposing individuals of *A. eugenii* to different concentrations of both extracts.

In mortality tests, the four evaluated concentrations of *C. odorata* extract did not reflect significant difference, it is observed that they have mortality effect on *A. eugenii*, the mortality rate between 20 and 30% of exposed insects; however, significant difference is observed when comparing the four concentrations with the witness. This is consistent with what Cobeña (2015) [17] reported as it evaluated six concentrations of *C. odorata* extract to control the green aphid (*Myzus persicae*), of which it found no significant differences in the range of concentrations that he evaluated (1% to 6%). They only found differences between the rallies when he compared them to the witness. Considering the time factor the results obtained are newly matched by Cobeña (2015) [17], from 24 hours the number of dead insects increases significantly.

On the other hand, Sánchez *et al.* (1996) [18], reported that an oily extract extracted from *C. odorata*, diluted to 5%, has a mortality effect of 95% of the individuals exposed to Bean Chrysomolide (*Andrector ruficornis*), this result is consistent with the mortality range that reflected the concentrations evaluated in this experiment, with the concentration of 2% of the ethanol extract being the closest.

In the bioassay of the *C. odorata* extract no significant differences were found considering the factors concentration and time, it was shown that this extract has low repellent effect, as determined by the index of Repellency. The repellent bioactivity of *C. odorata* is consistent with what Loayza reported (1982) [19] because when assessing the repellency of this plant species in the face of the termite attack (*Cryptotermes brevis*) we observed the *C. odorata* have the mayor effects, this suggest that the presences of terpenes in the woody tissues have repellent properties. It also notes that this plant tissue has a large amount of flavonoids, these are compounds with taste or bitter taste that makes them repulsive to consumption.

Significant differences were found in the mortality results of *D. regia* in the four concentrations evaluated. *D. regi*a extract was observed to have a mortality effect on *A. eugenii* from 76% to 85% on individuals exposed in the test. This is consistent with what was reported by Mekonnen and Haile (2015) [20], where they evaluated an oleic extract from *D. regia* seeds, the concentrations they evaluated are 1%, 2.5%, 5% and 10%. In the first three concentrations no significant

differences were found and reflected a mortality range of 75% to 88% of individuals of *Blattella germanica* exposed to such an extract. With regard to the time factor, significant differences were found in all the time measures evaluated, this is consistent with what Mekonnen and Haile (2015) [20] reported, where it is observed that as the hours pass exponentially increases the number of dead individuals.

The results obtained in the experimental phase of this research demonstrate the importance and benefits of the production and use in agriculture of plant extracts as biocontrollers of pests. This is reflected in higher and better product quality and thus the improvement of the quality of life of the producer, consumer and conservation of the environment.

The use of plant extracts as biocontrollers still require a lot of research to be applied effectively in integrated pest management. In the future, when methodological processes are perfected, the use of extracts will become more important than the one currently possessed [21, 22] (Silva *et al.*, 2002; Brechelt, 2004).

Conclusion

The results obtained with *D. regia*, shown a promising effect on mortality, mainly in the early hours of exposure to adults of *A. eugenii*.

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