Article

Biorational insecticide against corn rootworm in Durango

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Abstract

Agriculture in Mexico has surpassed the rational use of chemical insecticides, thereby impacting soil structure and the agricultural ecosystem in general. A pest control alternative is the use of toxic plants, which provide chemicals that modify the behavior of insects. The objective of this research was to evaluate the efficacy of a biorational insecticide generated with *Datura metel* Linnaeus applied on adult populations of the corn rootworm *Diabrotica undecimpunctata* Howardi during the 2019 spring-summer cycle, in Durango. One thousand six hundred twenty individuals were evaluated at different doses 10, 20, 50 and 70 mg L^{-1} every 0, 2, 4, 6, 8, 10, 12 and 24 h. In addition, the values of LD_{50} and the 95% confidence interval were determined for each treatment. The doses evaluated caused the mortality of corn rootworm, which varied depending on the dose and structure analyzed. The LD_{50} calculated for the root was 23.2 mg L^{-1}, for the leaf 34.1 mg L^{-1} and for the stem 89.3 mg L^{-1}. By increasing the dose to 70 mg L^{-1} of root, it was possible to achieve a mortality of 76%, so it is concluded that there is a directly proportional relationship between the structure evaluated and the dose applied to adults of corn rootworm and the time of exposure.

Keywords: corn pest, median lethal dose, plant extracts, toloache.

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Introduction

In Mexico, around 750 crops are produced nationwide, where corn is considered a basic element. There is a projection of corn production volume of 174 million tonnes of food, and it establishes that in the period from 2016 to 2030 it will increase 4.7%, which makes the Mexican countryside more susceptible to pest attack (SAGARPA, 2017). Most farmers are considered within a scale of low to medium production and most guide themselves only by a control technique, which has encouraged the indiscriminate use of pesticides, omitting the different control alternatives offered at the national level, such as integrated pest management (Zepeda-Jazo, 2018).

In the period from 2014 to 2017, the use of herbicides increased from 61.7% to 66.9% and insecticides from 45.3% to 54.8% (INEGI, 2017), which leads to a loss of fertility in soils and pollution of agricultural ecosystems. Fernández *et al*. (2013) mention that corn production in Mexico is always visualized with a political, social and economic perspective, hindering the management of the crop; however, despite the above, production has increased as the decades pass, from 1961 with 1.1 t ha^{-1} to 2012 with 3 t ha^{-1} (Cadet-Díaz and Guerrero-Escobar, 2018).

The crop of corn is affected by various pest insects and diseases that directly disturb growth (Hernández-Trejo *et al*., 2018). Pests cause damage from the moment of sowing since there are factors that favor their appearance, such as: climate conditions, preparatory work of the land, crop rotation and weed control, among others (Fernández *et al*., 2013).

According to Tinsley *et al*. (2012), for every root node injured by corn rootworm larvae, a yield loss of approximately 15% can be expected, which coincides with Subramanian *et al*. (2016), who claim that the corn rootworm has a devastating impact on crops. Corn pests are classified according to the affected area as soil, foliage, ear, grain, and post-harvest pests, each causing specific damage to the plant. Currently, about 70 species of pest insects are reported for corn, including the corn rootworm (*Diabrotica undecimpunctata*) (Deras, 2020).

There are different methods of pest control, prevailing, above all, broad-spectrum and fast-acting chemical insecticides, which increase the pollution of agricultural ecosystems, the loss of natural enemies and even the deterioration of human health, in addition to the fact that irrational use causes resistance in pest insects (Hernández-Trejo *et al*., 2018). Therefore, the use of biorational insecticides is considered a viable alternative for pest control because they decrease the population of insects without causing problems to the environment. Plant extracts are a new trend for the management and control of pest insects of various crops (Landivar-Ortíz *et al*, 2017).

One of the toxic wild plants of Mexico is *Datura metel* Linnaeus, which has insecticidal, herbicidal, antifungal, antibacterial, anticancer, anti-inflammatory, and anti-rheumatoid activity, in addition to being rich in alkaloid compounds (Monira and Munan, 2012). This plant has several bioactive components such as saponins, alkaloids, steroids, tannins, flavonoids, and triterpenoids (Dhawan and Gupta, 2017). Alkaloids can cause death by starvation or poisoning (Flores-Villegas *et al*., 2019). Therefore, the present research aimed to evaluate the efficacy of *D. metel* for the control of adults of the corn rootworm *D. undecimpunctata*, in Durango.

Materials and methods

The present work was carried out in the laboratory of the Polytechnic University of Durango. One thousand six hundred twenty adult individuals of *D. undecimpunctata* were collected, which were deposited in plastic boxes 30 cm long by 10 cm high and 20 cm wide. The insects were collected in the municipality of Vicente Guerrero, Durango, located at coordinates 23º 44’ 03” north latitude and 103º 59’ 12” west longitude. The plant material was collected in the municipality of Nombre de Dios, Durango (23° 50’ 54” north latitude, 104° 14’ 38” west longitude), consisting of 20 plants of *D. metel*, which were dried for a week at room temperature (25 ° C) and in the shade

Subsequently, leaves, stem and root were separated, then they were homogenized to a mesh size of 2-3 mm in a Thomas-Wiley Miller-type mill. To obtain the extract by maceration, 250 g of dry matter from each part of the plant was placed in 500 ml of methanol (2:1 ratio) (Flores-Villegas *et al*., 2019) and was kept at room temperature with periodic stirring for 24 h. Subsequently, the collected methanolic extract was concentrated in a rotary evaporator (D 404-2, Prendo) at 40 °C under reduced pressure.

To determine the insecticidal activity of each structure of *D. metel*, dilutions were made applying the method of maximum and minimum (Pulido and Cruz, 2013), which allowed identifying the doses (10, 20, 50 and 70 mg L^{-1}), these doses were sprayed on 30 *Diabrotica* adults in each plastic box, with each box constituting an experimental unit. The application was done uniformly using a spray bottle. In addition, there was a control group to which distilled water and 70% alcohol were applied. Mortality readings were performed at 0, 2, 4, 6, 8, 10, 12 and 24 h after the application of the extracts. The insect that, placed on a filter paper, did not have locomotor activity of its own was considered dead, either spontaneously or when stimulated with a brush or tweezers.

To ensure that the mortality values obtained are exclusively due to the lethal effect of the product evaluated, the following formula by Abbott (1925) was used, in which: corrected mortality (%)= $\frac{\text{(treatment mortality (\%)-control mortality (\%)x100)}}{\text{100-control mortality (\%)}}$ 1) and % mortality= $\left(x-\frac{y}{x}\right)$ x100. Where: y= dose and x= treatment (1= leaf, 2= stem and 3= root).

The statistical study and calculation of the LD_{50} were made with the PCS Farm program, to which the different doses and response levels, in this case mortality, for each one, are entered. The program performs a linear regression given by the probit-log relationship of the dose according to the numerical graphical method modified for computers of Lichfield and Wilcoxon, which delivers the LD_{50} and the 95% confidence interval (Cubillos *et al*., 1999). The differences between the LD_{50} were determined using X2 of trend (Epi Info 5.0 software).

Results and discussion

It was found that the LD_{50} of *D. metel* extract for *D. undecimpunctata* in adult stage depends on the plant part from which the extract was obtained, requiring less quantity when it comes from the root, followed by the leaf extract and finally, the stem extract for which a considerably larger volume is required (Table 1).

Table 1. Median lethal dose caused by extracts of different plant parts of *Datura metel* applied on *Diabrotica undecimpunctata*.

Vegetable parts	LD/LC (mg L^{-1})	95% Fiducial CI	
		Lower	Upper
Leaf	34.1	22.4	51.8
Stem	89.3	40	199.4
Root	23.2	14.6	37

The concentration-mortality results described in the probit model were adequate and indicate that the logarithmic transformation is the best to describe the mortality values of *D. undecimpunctata* due to the concentration of *D. metel* at different doses and times, which coincides with what was described by Bhusal *et al*. (2020), where they mention that the probit analysis is the one that best represents the mortality percentages. Figure 1 shows the fitted regression lines showing the linearity of the data with respect to the concentrations of each analyzed part (leaf, stem, and root) of *D. metel*.

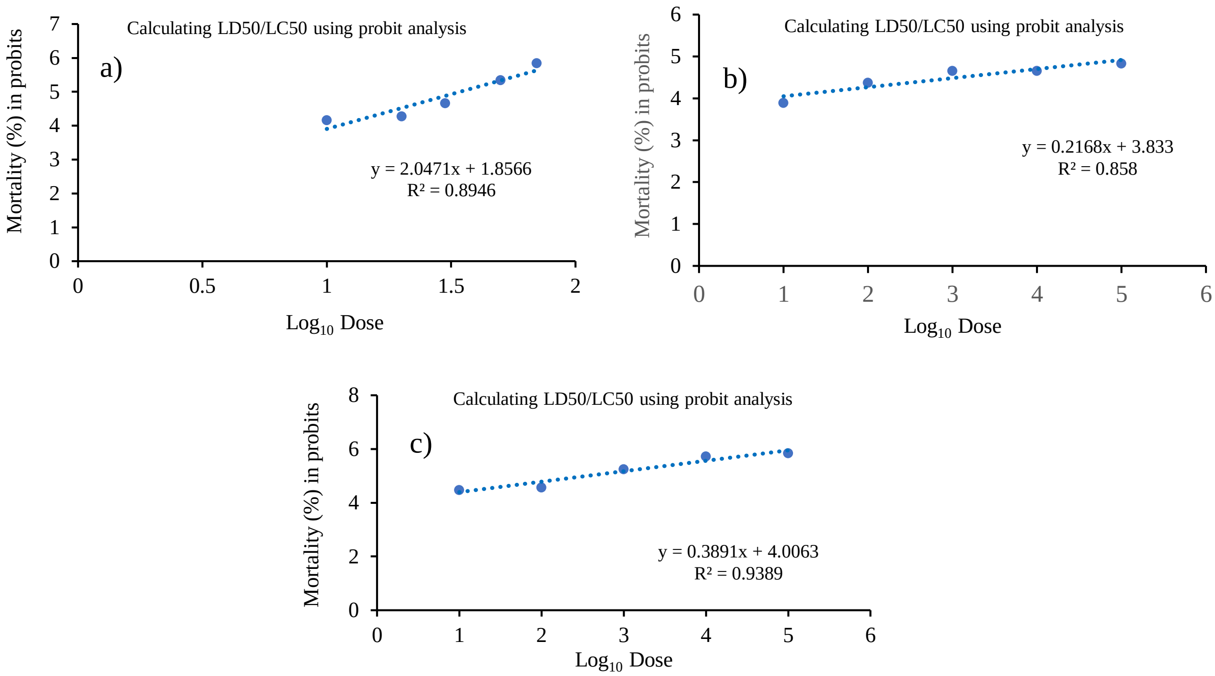


Figure 1. Predicted log-probit transformation of dead corn rootworm (*Diabrotica undecimpunctata*) adults at different doses of *Datura metel* extract for leaf (a), stem (b) and root.

The results obtained of leaf, stem and root at different doses presented different effects and high mortality in *Diabrotica* adults, similar to what was reported by Flores-Villegas *et al*. (2020), where they mention that a mortality of 86% can occur when using *D. metel* root. The toxicity observed by *D. undecimpunctata* is mainly due to the fact that the alkaloids present in the plant act locally or at a distance and mainly deter the insect from flying, perching on it, feeding or ovipositing (Bustos *et al*., 2017). It should be noted that, at present, biopesticides have shown an increase in their global participation in the management of pest insects and mites (Del Puerto *et al*., 2014). For the control of the corn rootworm *D. undecimpunctata*, bacteria and fungi such as *Bacillus thuringiensis* Berliner and *Beauberia bassiana* Bals have been mainly applied (Lemus-Soriano, 2017).

In the statistical analysis, significant statistical differences were observed between leaf, stem, and root extracts (Table 2). The highest percentage of mortality was found with extracts obtained from the root, coinciding with the calculated LD_{50}. Organic insecticides generated from toxic plants reduce populations of the corn rootworm *D. undecimpunctata* (Nzinga *et al*., 2016) to populations below their economic threshold, which allows minimizing the use of synthetic pesticides, in addition to the fact that most organic insecticides that have been applied in the field are usually not phytotoxic because the substances with these effects are eliminated in the application process (Bustillo, 2008).

Table 2. Test of significant difference between treatments (extracts from three structures of *Datura metel* on *Diabrotica undecimpunctata*).

Number	Treatment	(%) mean mortality	1	2
2	Stem	35.83	****	
1	Leaf	42.7	****	
3	Root	56.6		****

When applying the crude extracts on adult insects of *D. undecimpunctata*, the toxic effects in them began to be observed immediately after the application, however, for the stem extract the effects occurred after 4 h of application. In addition, a deterrent effect was observed in the rootworm, which means a deterioration in the primary feeding of the insect, which causes increased mortality (Esparza-Díaz *et al*., 2010). On the other hand, in the generalized linear model, mortality behavior was observed with an adjustment coefficient of 0.89 (Table 3).

Table 3. Sum of squares test (percentage of mortality of *Diabrotica undecimpunctata*).

Dependent variable	Multiple regression R	Multiple regression R^2	R^2 adjusted	SS Model	df	P
(%) mortality	0.95105	0.90514	0.89219	2163.2	4	0
Dependent variable	MS model	SS model	df residual	MS residual	F	P
(%) mortality	5409.424	2284.217	31	73.68443	73.41339	0

The univariate test of importance (Table 4) with a significance level $\alpha = 0.05$ shows that the dose and type of treatment (doses 10, 20, 50 and 70 mg L^{-1}) present significant differences ($p < 0.05$); that is, they are important factors for the increase in mortality of *D. undecimpunctata*.

Table 4. Univariate test of importance for the percentage of mortality of *Diabrotica undecimpunctata* using different doses of *Datura metel* extract.

Effect	SS	Degrees of freedom	MS	F	P
Intercept	1114.827	1	1114.827	15.12975	0.000496
Treatment	1157.407	2	1157.407	15.070763	0.000405
Treatment^2	1543.21	2	1543.21	20.9435	0.000072
Dose	3876.579	3	3876.579	52.61056	0
Dosage^2	677.765	3	677.765	9.19821	0.004865
Error	2284.217	6	73.684		

Table 5 shows the statistical differences between the different doses applied 10, 20, 50 and 70 mg L^{-1} of *D. metel* on adults of *D. undecimpunctata*. In addition, the model shows that, by increasing the dose to 70 mg L^{-1} of root, it was possible to reach a mortality of 76%. According to Yaranga (2015), the biotoxic effect of *D. metel* is probably related to the synergistic activity of alkaloids, triterpenes, some types of phenols and tannins and to the complexity of trace products.

Table 5. Least significant difference test for the different doses applied of *Datura metel* on adults of *Diabrotica undecimpunctata*.

No.	Dose (mg L^{-1})	(%) Mean mortality	1	2	3	4
1	0	10			****	
2	10	30	****			
3	20	33.88	****			
5	50	69.44				****
6	70	76.66		****		

Equation 1 expresses the behavior of mortality of *D. undecimpunctata* using different doses and treatments: $\% \text{ mortality} = 48.5478 - 51.2582x + 1.4055y + 13.8889x^2 + 0.0882xy - 0.009y^2$ 1). Where: y= dose and x= treatment (1= leaf, 2= stem and 3= root). Figure 2 shows the increase in mortality of *D. undecimpunctata* using different doses and treatments. The root and doses above 50 mg L^{-1} were used, it is possible to increase mortality up to 76%.

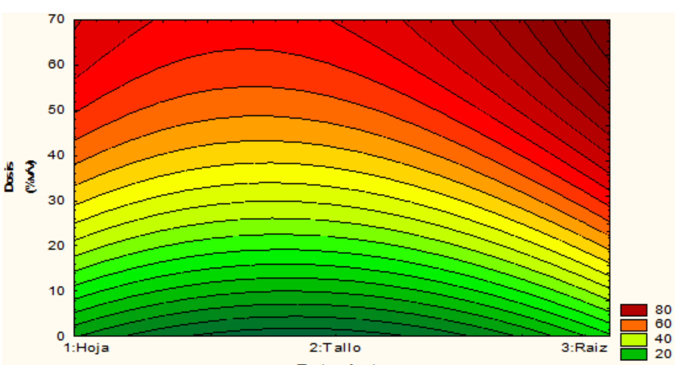
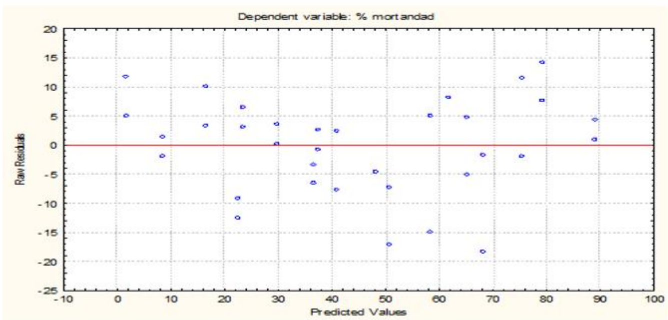


Figure 2. Contour plot for mortality of *Diabrotica undecimpunctata* using extracts of *Datura metel* in leaf, stem, and root with different doses.

The model was validated, taking into consideration the assumptions of normality of residuals, homogeneity of variance and independence of the predicted values against residuals. Figure 2 of predicted values vs residuals (Figure 3a) shows random behavior. The homogeneity of variance test (Figure 3b) shows that the residuals have a normal distribution, Kolmogorof-Smirnof, Lilliefors and Shapiro-Wilk tests $p > 0.5$.

a)



b)

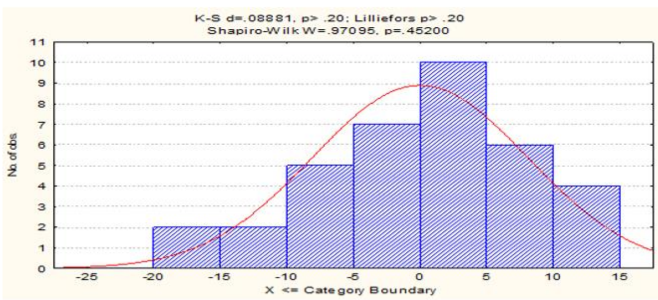


Figure 3. a) homogeneity of variance test and predicted values graph; and b) normality test.

The independence of the residual values and the predicted values of the model was verified by means of their correlation (Table 6), concluding that they are independent with $p < 0.05$.

Table 6. Test of correlation between predicted and residual values of the model.

Variable	Predicted (pH)	Residual (pH)
Predicted (pH)	1	0
Residual (pH)	0	1

Conclusions

All treatments significantly reduced the adult population of corn rootworm. The effects of the structures evaluated showed a high mortality rate of up to 76%. Also, that there is a directly proportional relationship between the structure evaluated and the dose applied to adults of the corn rootworm *D. undecimpunctata* and the time of exposure. The extract that requires the highest dose of application is that from the stem and the one that requires less dosage is the root extract. The production of biorational insecticides offers the possibility of reducing the deterioration of ecosystems and benefiting the entomofauna of the environment.

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