Ascending polarity extracts of *Dodonaea viscosa* leaves on instars of *Spodoptera frugiperda*

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Abstract

The search for environmentally friendly alternatives for the control of agricultural pests, which avoid damage to public health and the surrounding ecosystem, is a current demand that deserves responsible bioethical attention. In this sense, the objective of the present work was to evaluate the activity of the extracts of *Dodonaea viscosa* leaves with ascending polarity on the development of *Spodoptera frugiperda*. Foliar extracts of *D. viscosa* were obtained by cold maceration, using solvents in ascending polarity: hexane, dichloromethane and methanol, which were used in the antifeedant tests to know their individual effect. An experiment was conducted with two antifeedant bioassays, one with an artificial diet and the other with corn leaves; in both, the three extracts of *D. viscosa* obtained were applied at a concentration of 1% m/v. The experiment was carried out with L3 larvae of the third generation of *S. frugiperda*, from which the bioassays were carried out, during all stages of development. The data were subjected to a multifactorial Anova and to the comparison of means (Tukey $p= 0.05$). A principal component analysis (PCA) was performed to identify the variables influenced in each stage of the insect. The variables with significant differences were larval mortality, duration of the larval and pupal stage; as well as pupal weight and number of eggs laid. The extracts analyzed showed effects on the interaction with the type of diet, which was reflected in the variables evaluated during the development of *S. frugiperda*.

Keywords: *Spodoptera frugiperda*, alternative pest control, botanical products, Lepidoptera: Noctuidae.

Introduction

Corn is the highest-produced agricultural cereal in the world after wheat (Zermeño-González *et al*., 2015). However, it is seriously affected by several factors that decrease its yield and development, including diseases or insect damage (Ayala *et al*., 2013; Reséndiz *et al*., 2016). *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) is a pest native to America; it causes great damage to this crop (Kalleshwaraswamy *et al*., 2018; Salazar-Blanco *et al*., 2020), the larval stage being the most harmful since it is when it feeds on the tender shoots of the plant, on many occasions penetrating the buds (Salas-Araiza *et al*., 2018), generating significant losses in grain production (Toma *et al*., 2017; Ramírez *et al*., 2018).

This insect has generated resistance to a wide variety of synthetic chemicals used for pest control. Faced with this problem, chemical insecticides with short-term effect are used for its combat and control, resulting in high harm due to their high toxicity to human health (Del Puerto *et al*., 2014), microorganisms, pollinators, fish, as well as water and soil quality (García *et al*., 2012), in addition, larvae develop resistance to these chemicals and it is increasingly difficult to control them (Giraudo *et al*., 2015). To address this problem, alternatives with natural products have been proposed for the control of insects, using plant extracts (Tembo *et al*., 2018), that reduce the impact on public health, other beneficial insects and therefore the environment adjacent to the cultivation sites (Carvalho, 2017).

These extracts frequently cause sublethal effects on insects, that is, they cause physiological or behavioral changes that deplete the physical state of the insect and can have unfavorable effects on the entire population; some damages may be inhibition of larval growth and development, as well as alterations in behavior (antifeedant or repellency) (Rosales-Juárez *et al*., 2015). Some plant extracts such as: *Melia Azedarach* (L.), *Ricinus communis* (L.) and *Annona muricata* (L.) have been evaluated for the control of *S. frugiperda* (Ángel-Ríos *et al*., 2015), showing a lethal effect on larvae of 72% and on pupae of 40-80%.

Dodonaea viscosa (L.) Jacq. (Sapindaceae) is a resinous shrub that is distributed throughout much of the world, including Mexico (Rzedowski, 2006; Díaz *et al*., 2015). It develops in different types of vegetation, such as in tropical deciduous, evergreen forest, oak forest, secondary communities and deteriorated shrublands, it has a great adaptability to unfavorable conditions and a high capacity to regenerate after anthropic disturbances (González-Elizondo *et al*., 2012).

It has been reported that this shrub has great phytochemical potential, as well as various uses in traditional medicine, such as anti-inflammatory, healing, cytotoxic, antidiabetic, etc. (Mora *et al*., 2017), in this framework, it is considered to be non-toxic to the consumer and at the same time an eco-friendly and harmless alternative with beneficial insects (Mesa *et al*., 2019), as well as defense against attacks and in the implementation of pest control (Sotelo-Leyva *et al*., 2020).

In this sense, the secondary metabolites of *Dodonaea viscosa* have been attributed effects with bioinsecticidal activity (Díaz *et al*., 2015; Sotelo-Leyva *et al*., 2020); such as phenolic compounds, flavonoids, alkaloids, terpenes, tannins and saponins (Shafek *et al*., 2015; Al-Snafi, 2017), which has demonstrated its toxic and repellent effect on various insects and mites (El-Gengaihi *et al*., 2011; Sotelo-Leyva *et al*., 2020). As well as effects of the biological activity of different leaf

extracts of *D. viscosa* against various insects such as *Epilachna paenulata*, *Spodoptera littoralis*, *Myzus persicae*, *Rhopalosiphum padi* and *Melanaphis sachar* (Scapinello *et al*., 2014; Díaz *et al*., 2015; Sotelo-Leyva *et al*., 2020).

The evaluation of a fraction of a rutin flavonoid extracted from the leaves of *D. viscosa*, evaluated by antifeedant tests in *S. frugiperda* larvae, is also documented, the results showed alteration on the lifetime of the insect (Silva *et al*., 2016), but the effects through extractions in ascending polarity on the development of *S. frugiperda* are unknown.

Therefore, it is considered that most of the secondary metabolites are stored in greater quantity in leaf tissues and that it facilitates the functionality of these compounds present by maceration with solvents of different polarities, which implies that the chemical composition of interaction facilitates a better response, so it was proposed as an objective to evaluate the activity of extracts of *D. viscosa* leaves in ascending polarity on the development of *S. frugiperda* under artificial diet and natural corn leaf diet.

Materials and methods

Collection of plant material of *D. viscosa*

Two kilograms of leaf sample of *D. viscosa* were collected in August 2017, in the locality of Las Trojes, Jocotepec, Jalisco, Mexico (-103.32 longitude, 20.33 latitude) (Figure 1).
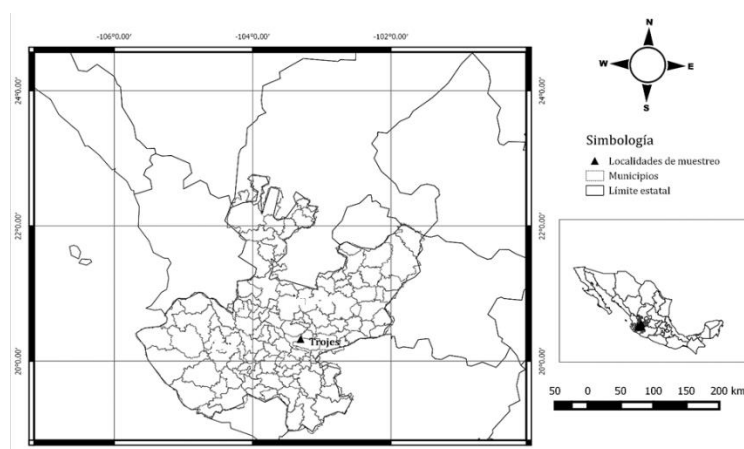

Figure 1. Site and collection of plant material of *D. viscosa*. Las Trojes, Jocotepec, Jalisco, Mexico.

Preparation of the extract

Five hundred grams of *D. viscosa* leaves were weighed, dried at room temperature for 15 days and pulverized. Cold maceration was used, using 500 ml for each solvent in ascending polarity (hexane, dichloromethane and methanol), subsequently, they were filtered with Whatman® No. 1 paper and evaporated in a rotary evaporator (brand R II - SJ24/40, A, 100-120V - BUCHI) until obtaining a solid extract for each solvent according to Nawaz *et al*. (2020), which were stored in amber bottles and stored in refrigeration until the evaluation of the bioassays. Dilutions were prepared at a 1% concentration per treatment with Tween 80 at 0.1% as a surfactant (Rodríguez-Soto *et al*., 2018) and for control only Tween 80 was used.

Field collection of *S. frugiperda*

During July and August 2018, manual collections of *Spodoptera frugiperda* larvae were carried out in corn cultivations, at the University Center for Biological and Agricultural Sciences of the University of Guadalajara, located with the coordinates (CUCBA) (20° 44' 47" north latitude 103° 30' 43" west longitude).

Fall armyworm breeding

The collected larvae were transferred to the laboratory, where the brood was kept in an incubator, at a relative humidity of 60%, 25 °C ±2 °C and a photoperiod of 12:12 h of light, they were fed on corn leaves until the formation of the pupa. In the pupal stage, they were sexed according to Guzmán (2016), using a stereoscopic microscope (Olympus Optical, model SZ-40/SZ-ST, 10); subsequently, they were passed to 1-oz cups until the emergence of adults. After the emergence of moths, adults were placed in brown paper bags of 50 x 20 cm for copulation and oviposition, they were fed with a 10% honey solution. Egg layings were identified, which were collected daily for 10 days in a row. The breeding of the insect was maintained until obtaining neonatal larvae of the third generation.

Antifeedant tests

In bioassay 1, the larvae were fed with an artificial diet (Poitout and Bues, 1974) and in the second bioassay, they were fed with corn leaves (cultivar LUG 282), this in order to have greater certainty or evidence of the effect of the extracts. The artificial diet was cut into pieces of approximately 1 cm^3 and the corn leaves were cut into pieces of 2 cm^2. Both diets were impregnated by immersion with each of the foliar extracts at the 1% concentration dissolved in Tween 80.

In order to determine the amount of extract retained in both bioassays, it was weighed before and after being immersed in the extract. The antifeedant tests consisted of feeding 100 L3 larvae per treatment, individualized in 1-oz cups, placing a piece of diet, previously impregnated with the extracts. The antifeedant evaluation was carried out 24 h after leaving the larvae in starvation. The parameters evaluated were mortality and weight of larvae every 24 h for seven days, pupal weight, duration of larval, pupal and adult stages, sex ratio, number of eggs laid (the outermost egg layers were counted and multiplied by the number of layers) and hatched (Callado-Galindo *et al*., 2013), adult emergence, pupae.

As well as food consumption (calculated using the formula initial weight of the diet (mg) by diet consumed between 100), relative growth rate RGR= (FW-IW)/(AW x T), where FW is the final weight of the larvae (mg), IW is the initial weight of the larvae (mg), AW is the average weight of larvae during the essay (mg) and T is the duration of the feeding period (7 days). This parameter represents the gain (mg) of biomass of the insect per day in relation to its weight (g), methodology modified by Scriber and Slansky (1981) cited by Valencia *et al*. (2014).

Determination of the content of total phenols and flavonoids

The total phenol content was determined and quantified according to the method proposed by Waterman and Mole (1994) modified by Bernabé-Antonio *et al*. (2017), the concentration of total phenols in extracts was measured by spectrophotometry, based on a colorimetric oxidation-reduction reaction. Absorbance was measured at 760 nm. The results were expressed in mg of gallic acid per gram of extract (mg GA g^{-1} extract). Each sample was analyzed in triplicate. The quantification of flavonoids was performed according to Chang *et al*. (2002) modified by Bernabé-Antonio *et al*. (2017). The benchtop UV spectrophotometer was used for absorption at 7 300 nm. The results were expressed as milligrams of quercetin equivalents per gram of dry weight (mg cat g^{-1} extract).

Experimental design and statistical analysis

The experimental design was completely random. All the results of the measured variables were evaluated using a multifactorial analysis of variance and were subjected to the Tukey test *p*= 0.05 for the homogeneity of groups between treatments. The variables evaluated were grouped by phase of the insect, in the larval stage: duration of the larval stage (days), larval weight (mg) and percentage of larval mortality; in the pupal phase: duration of the pupal stage (days), pupal weight (mg), sex ratio, deformed and non-hatched individuals; in the adult stage: duration of the adult stage (days), deformed adults, number of eggs laid and percentage of eggs hatched, grouped in treatments as observed in Table 1. The analysis was performed with the Minitab 16 program (Minitab® statistical software). In addition, they were subjected to a principal component analysis (PCA), for the incidence of the most important variables in both bioassays.

Table 1. Experimental design of the treatments of *D. viscosa* leaves.

Solvent	Treatments			
Leaves	Control	Hexane	Dichloromethane	Methanol
Bioassay 1 artificial diet	T1	T2	T3	T4
Bioassay 2 natural diet	T5	T6	T7	T8

Bioassay 1= artificial diet food; T1= control; T2= Hexane; T3= Dichloromethane; T4= Methanol. Bioassay 2= natural diet food (corn leaves); T5= control; T6= Hexane; T7= Dichloromethane; T8= Methanol.

Results and discussion

Effect of extracts on the fall armyworm *S. frugiperda*

The percentage of larval mortality showed significant differences between the treatments (F= 4.52, *p*= 0.05); the highest mortality occurred in the natural diet in T7 and T8 with 36.6 ±4.08 and 33.3 ±4.08 % respectively (Table 1). These larval mortality values are lower than those reported by Salinas-Sánchez *et al*. (2020), where they evaluated by antifeedant tests the effect of an aqueous fraction of *Serjania schiedeana* (Sapindaceae) at concentrations 250, 500, 1 000, 1 500 and 2 000 ppm, the results reflected the highest larval mortality (72%) at the concentration of 250 ppm. For their part, El-Din and El Gengaihi (2000) report 90% of larval mortality, caused by plants

belonging to the family Sapindaceae, evaluated by antifeedant tests in *Spodoptera litoralis* larvae; therefore, the mortality rate observed due to the effect of the application of the extracts could be due to the presence of some secondary metabolites in the extracts, such as terpenes.

Table 2. Effect of the leaf extracts of *D. viscosa* on the variables evaluated in the different stages of development of *S. frugiperda*.

Treatments	Larval mortality (%)	Duration of the stages (days)		Pupal weight (mg)	No. of eggs laid
		Larva	Pupa		
Bioassay 1 artificial diet					
T1	10 ±4.08a	14.7 ±0.32a	9.8 ±0.2ab	0.211 ±0.006c	345.46 ±26.15b
T2	26.6 ±4.08ab	14.9 ±0.32a	9.3 ±0.2a	0.204 ±0.006bc	203.83 ±26.15c
T3	20 ±4.08ab	15.5 ±0.32ab	9.3 ±0.2a	0.195 ±0.006abc	137.66 ±26.15c
T4	23.3 ±4.08ab	16.1 ±0.32ab	9.9 ±0.2ab	0.211 ±0.006c	120.2 ±26.15c
Bioassay 2 natural diet					
T5	16.6 ±4.08ab	17.1 ±0.32b	11 ±0.2b	0.186 ±0.006ab	567 ±26.15a
T6	26.6 ±4.08ab	16.1 ±0.32ab	11 ±0.2b	0.195 ±0.006abc	215.33 ±26.15c
T7	36.6 ±4.08b	16.3 ±0.32ab	10.6 ±0.2b	0.181 ±0.006a	450.3 ±26.15b
T8	33.3 ±4.08b	17.1 ±0.32b	10.9 ±0.2b	0.184 ±0.006a	211.6 ±26.15c

Multivariate Anova, Tukey test α= 0.05. Means with different letters are statistically significant; ±= standard error.

In the time of development of the larval stage, there were significant differences between the treatments (F= 7.22, *p*= 0.0001), this according to the Tukey test, in the artificial diet, T2 registered the shortest time 14.9 ±0.32, as well as T1-14.7 ±0.32 and the longest time occurred in the natural diet in T5 and T8, with averages of 17.1 ±0.32 days for both treatments (Table 2). With respect to pupal weight, a decrease in weight was observed, according to the statistical analysis (F= 8.81, *p*= 0.01), in the natural diet in T7 and T8 0.181 ±0.006 and 0.184 ±0.006 mg, respectively; compared to the artificial diet, T1 and T4, which showed an average weight of 0.211 ±0.006 mg (Table 2). It should be noted that, to estimate the quality of diets or food, the weight of larvae and pupae is a fact that must be considered (Cohen, 2015).

For this reason, it is considered that a greater weight of larvae can ensure effective survival. In this sense, corn leaves are composed of parenchymal cells, lignin, cellulose, etc. and provide the same nutrients as the artificial diet, this is rich in carbohydrates, being superior to corn leaves, in addition, it contains as ingredients wheat flour, wheat germ, agar, yeast, sugar, ascorbic acid, vitamin C, the latter acts as a food stimulant and in different defensive reactions such as the antioxidant (Cohen, 2015), it is for that reason that the highest weights occurred in the artificial diet.

The duration times of the pupal stage showed significant differences according to the analysis (F= 8.47, *p*= 0.002). In the artificial diet, the shortest times occurred in T2 and T3, with 9.3 ±0.2 for both treatments; however, in the diet with corn leaves, a longer time was observed with means of 11 ±0.2 in T5 and T6 (Table 2). In this sense, the duration time or variations in the larval and pupal stages of *S. frugiperda* may be due to the quality and type of food (Flores-Quinches *et al*., 2021). According to this, the life cycle of the fall armyworm is highly influenced by environmental conditions such as humidity, photoperiod and temperature, but in this case, the diet is the parameter that determines the duration of the phases.

On the other hand, the fertility of the insect also showed significant differences (F= 22.72, *p*= 0.0001), reducing the number of eggs. The lowest averages occurred in the artificial diet in treatments T2, T3 and T4, with averages of 137.66 ±25.15, 203.83 ±25.15 and 120.3 ±25.15, respectively. For the treatments used with natural diet, T6 and T8 were significant, with averages of 215.33 ±26.15 and 211.6 ±26.15 consecutively. While the highest number of eggs in the natural diet was in T5 and T7 (Table 2).

In this regard, Malarvannan *et al*. (2009) report the effect on other species of the family Sapindaceae, such is the case of *Dodonaea angustifolia*, their results showed an ovicidal effect on the insect *Helicoverpa armígera*, at doses of 0.5 and 1% in extracts of petroleum ether and acetone. However, Castillo *et al*. (2009) evaluated extracts of different organs of the plant, such as fruits, leaves and branches of *D. viscosa* of Uruguayan origin, none of these extracts showed to be active against the polyphage *Spodoptera littoralis* (Lepidoptera: Noctuidae).

In relation to this, a reduction in the number of eggs laid and hatched is due to the difficulty of assimilating nutrients during the larval period and is reflected in a reduced number of eggs (Arrese and Soulages, 2010). Therefore, these results indicate the presence of a sublethal effect of organic extracts on the insect, manifesting itself in stages after the larval stage, with the incidence of a reduction in the number of eggs laid.

Table 3 shows the results of the amount of food ingested or relative consumption rate (RCR), according to the analysis, similar diet consumptions occurred in both bioassays with averages of 0.317 ±0.05 to 0.49 ±0.15 mg mg^{-1} day^{-1} of consumption; however, it does not mean that the same assimilation of nutrients occurs, this is confirmed by the growth rate (RGR), in both bioassays, showing significant differences (F= 20.25; *p*= 0.001), the larvae with the greatest weight were in bioassay 1, with means of 0.514 ±0.02-0.609 ±0.05 mg mg^{-1} day^{-1}, while the lowest weights recorded were in bioassay 2, showing the lowest inhibitory effect with means of 279 ±0.07-0.31 ±0.03 mg mg^{-1} day^{-1}, respectively.

Table 3. Effect of extracts on growth and food consumption in *S. frugiperda* larvae.

	Bioassay 1 artificial diet			Bioassay 2 natural diet	
Treatments	Relative consumption rate (RCR) (mg mg^{-1} day^{-1})	Relative growth rate (RGR) (mg mg^{-1} day^{-1})*	Treatments	Relative consumption rate (RCR) (mg mg^{-1} day^{-1})	Relative growth rate (RGR) (mg mg^{-1} day^{-1})*
T1	0.324 ±0.05	0.609 ±0.05a	T5	0.49 ±0.15	0.279 ±0.07b
T2	0.353 ±0.03	0.555 ±0.07a	T6	0.497 ±0.06	0.34 ±0.04b
T3	0.317 ±0.05	0.514 ±0.02a	T7	0.486 ±0.02	0.356 ±0.01b
T4	0.364 ±0.04	0.604 ±0.07a	T8	0.322 ±0.1	0.31 ±0.03b

RCR= relative consumption rate; RGR= relative growth rate. Analysis of variance, homogeneity of groups in bioassay 2 (Tukey α= 0.05). *= means with different letters are statistically significant; ±= standard deviation.

Effect of the treatments with the greatest influence on the variables evaluated on the instars of the fall armyworm *S. frugiperda*

In this part, only the variables that had some effect on *S. frugiperda* were used in the antifeedant tests, according to the principal component analysis (Figure 2). In Figure 2 A, it is observed that the components that had eigenvalues greater than 1 are the duration of the larval stage with a

value of 1.37 and larval mortality with a value of 1, this according to the sedimentation graph, which indicates that the closer to 1, the greater the influence of the variable on the effect on the insect, in relation to this, it has been reported that the duration of the larval stage in this insect can prolong or shorten the cycle depending on the food it has; that is, the fall armyworm prolongs its cycle when it feeds on cotton and reduces it when it feeds on corn, under the same environmental conditions.
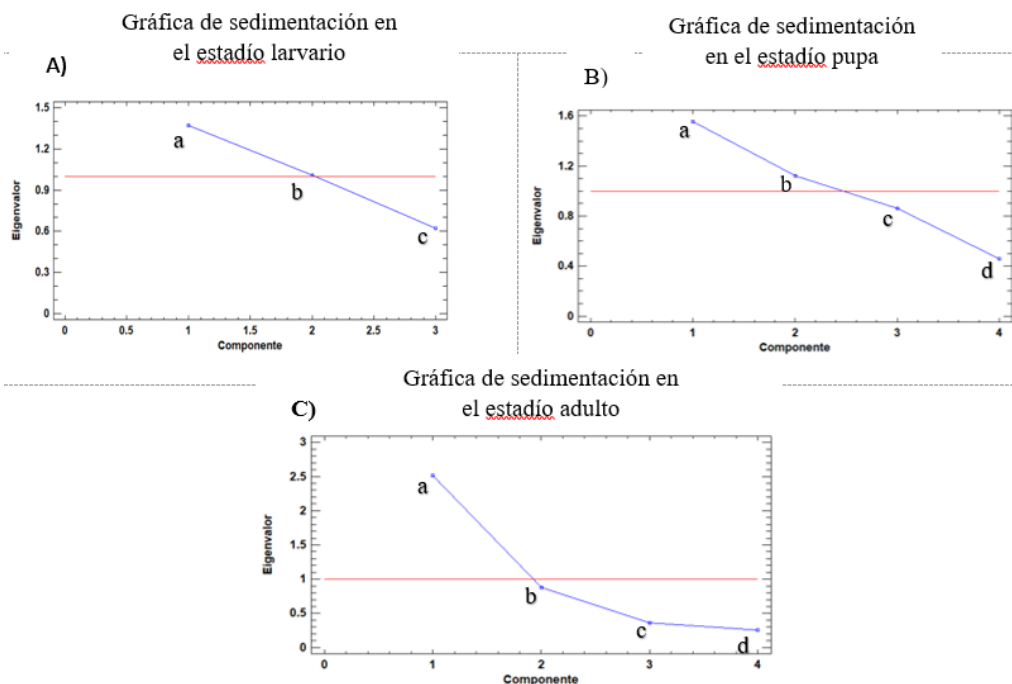

Figure 2. Sedimentation graphs of the principal component analysis, in the instars of *S. frugiperda.* A) larva: a) duration of the larval stage; b) larval weight; and c) larval mortality. B) pupa: a) duration of the pupal phase (days); b) pupal weight (mg); c) sex ratio; and d) deformed; C) adult stage: a) duration of the adult stage (days); b) deformed adults; c) the number of eggs laid; and d) percentage of hatched eggs.

On the other hand, the leaves as a food source favored the highest percentage of larval mortality, because the leaves were dehydrated shortly after being cut, thus hindering ingestion by the larvae. On the other hand, the artificial diet rich in flours allowed the absorption of the isolated compounds, on the food, they also allowed keeping constant most of the variables, such as temperature, amount of food, relative humidity, among others, which is advantageous for the development of the respective bioassays.

In the pupal stage, Figure 2 B shows two of the extracted components, they had values higher than 1, with a value of 1.55 for pupal weight and with a value of 1.12 for duration of pupal stage. This indicates that the insect is affected in its weight and duration, and it can generate deformed adults. The results of the principal component analysis for the adult stage of *S. frugiperda* are observed in Figure 2 C of sedimentation. For this case, only one extracted component obtained values greater than 1 (number of eggs laid), showing eigenvalues of 2.51.

Which indicates that adults are affected in their reproduction; that is, the extracts caused a sublethal effect on the development of the insect, caused by the intake of the metabolites impregnated in the diet, affecting fertility, with the presence of a significant reduction in the number of eggs laid in both experiments. Thus, these results coincide with those reported by Salama *et al*. (1988), who demonstrated that 5% hexane and chloroform extracts of *D. viscosa* leaves caused a considerable reduction of eggs laid up to 50% in *Spodoptera litoralis*. This effect may be due to the presence of phenol-type compounds, such as flavonoids present in its leaf tissue.

Relationship analysis of bioassays 1 and 2 in *S. frugiperda*

The tests for the evaluation of the extracts in both bioassays were subjected to the analysis with 12 variables. Figure 3 shows only those variables that did not have a correlation for both experiments. The effect on larval mortality was different in both bioassays, showing a greater impact on leaf-fed insects (bioassay 2), as shown in Figure 3A. This effect occurred in the same way in the duration of the larval stage (Figure 3B), in the duration of the pupal stage (Figure 3C) and the number of eggs laid (Figure 3E). However, for pupal weight, although in Figure 3D, greater impact is observed on individuals fed with artificial diet (bioassay 1), the lowest weights occurred in the leaf diet (bioassay 2).
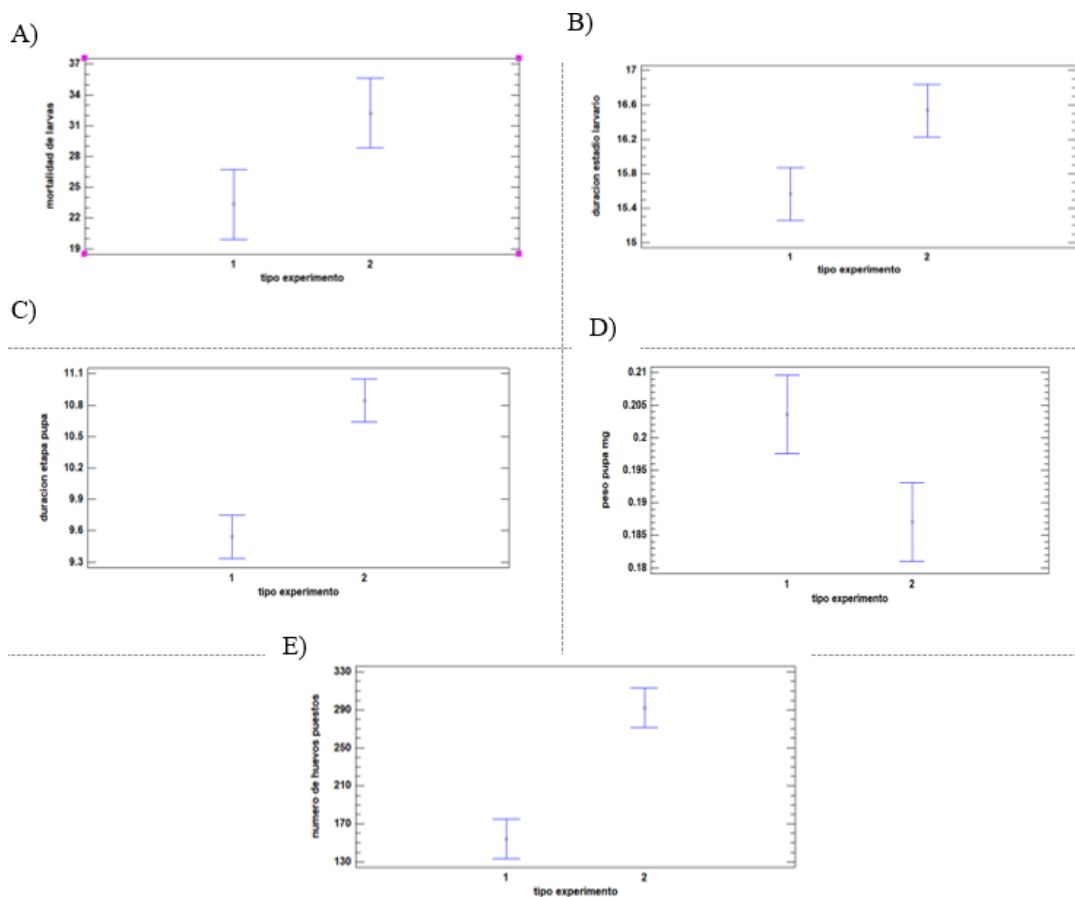

Figure 3. Multivariate analysis, means of the variables evaluated of the two antifeedant experiments. 1 artificial diet bioassay, 2 corn leaf diet bioassay. A) larval mortality; B) duration of the larval stage; C) duration of the pupal stage; D) pupal weight; and E) number of eggs laid.

In addition, the presence of phenols (15.58% 100 mg GA g^{-1} extract) and total flavonoids (1.33% 100 mg cat g^{-1} extract) was determined. So, the presence of these compounds indicates that this species is an important source with relevant biological activity.

There are reports of the various secondary metabolites present in *D. viscosa*, including alkaloids, saponins, flavonoids, terpenes, phenols, according to Mostafa *et al*. (2014), considered as possible responsible for the effect on the development of the insect. For their part, Schneider *et al*. (2017) evaluated the effect of neem (*Azadirachta indica*) through antifeedant tests on *Diatraea saccharalisa* F. (Lepidoptera: Crambidae). For their part, Niu *et al*. (2010) mention that a series of clerodane diterpene-type compounds with insecticidal biological activity against *Spodoptera exempla* and *S. littoralis* and prenylated flavonoids have been isolated from the aerial parts of *D. viscosa*, which have shown activity against lepidoptera (*Plutella xylostella* and *Pieris rapae*) and the beetle *Sitophilus oryzae*.

Conclusions

The extracts of *Dodonaea viscosa* leaves showed efficacy on *Spodoptera frugiperda*, by modifying its development and reproduction. Therefore, the use and application of products based on botanical extracts of *D. viscosa* turn out to be an interesting alternative for the control of agricultural pests in an eco-friendly way.

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