EFFICACY OF PLANT EXTRACTS AND NUTRITIONAL ALTERATIONS ON *Spodoptera frugiperda* (J.E. Smith) UNDER *IN VITRO* CONDITIONS

Supported by tetfund TERTIARY EDUCATION TRUST FUND

G. González-López[1], M. Ojeda-Chi[2], F. Casanova-Lugo[1], V. Díaz-Echeverría[1], A. Avitia-Deras[1], M. J. Soria-Fregoso[3], W. Cetzal-Ix[3] and S. K. Basu[4]

[1]Instituto Tecnológico de la Zona Maya, Tecnológico Nacional de México
[2]Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Yucatán, México
[3]Instituto Tecnológico de Chiná, Campeche, México
[4]Department of Biological Sciences, University of Lethbridge, Lethbridge AB Canada
*Corresponding author: rolito22@hotmail.com

Received: November 15, 2016 **Accepted:** January 25, 2017

Abstract: The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is the main pest of maize in the state of Quintana Roo, Mexico. Neem plant (*Azadirachta indica*L.) has been used for its control, with good results; however, it is necessary to evaluate new alternatives from other plant extracts, as well as environmental factors that can affect the amount of secondary metabolites. The objective of this present work was to evaluate the efficacy of aqueous extracts prepared from fresh leaves of *A. iIndica* (Ai), *Gliricidia sepium* (Gs) and *Leucaena leucocephala* (Ll), pure and combined for the control of *S. frugiperda* under *in vitro* conditions. Two bioassays were established and six treatments were evaluated: 1) Ai, 2) Gs, 3) Ll, 4) Ai + L1, 5) Ai + Gs, and 6) Ai + Ll + Gs and a control group. Treatments were applied at the beginning and half of the bioassay during a period of eight days. At the end of the bioassay growth and food consumption of the larvae, nutritional disorders, and the efficacy of the plant extracts it was measured. The lowest weight of the larvae treated with the extracts in comparison with the control. Also, the lowest food consumption was for the extracts of Ai, Ll, Ai + Ll, Ai + Gs and Ai + Ll + Gs, and the highest for the control.The larvae exposed to the treatments Ai + Ll, Ai + Gs and Ai + Ll + Gs presented a lower consumption index, larval growth and food utilization index in relation to the control group. The pure extracts of *L. leucocephala* and the combined *A. indica* with *L. leucocephala* increased efficiency compared to the other treatments. It is concluded that the best efficacy values were for extracts of *A. indica* with *L. leucocephala* and *L. leucocephala*. Therefore, they represent a good organic alternative for the control of *S. frugiperda* in the south of Quintana Roo.

Keywords: Consumption, nutrional alterations, *Azadirachta indica*, *Gliricidia sepium*, *Leucaena leucocephala*, *Zea mays*

Introduction

Maize (*Zea mays* L.) is a native plant of Mexico that is used for human food and livestock, in addition to its industrial use. Due to its productivity and adaptability, the cultivation of maize has spread all over the world. Mexico produces 2.7% of the total world maize (more than 24.95 million tonnes of maize in 2015), being a major producing country with higher consumption (SIAP, 2014). The volumes and quality of maize production in Mexico are limited mainly by the incidence of pests and diseases, as well as by abiotic factors such as nutrient deficiency in soils and drought. However, the maize yield decline due to pests is 20-30%, and can reach up to 60% in most crops (Moreno and Gonzalez, 2011).

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is one of the main pests of maize cultivation in tropical and subtropical regions of the Americas. In recent years, the increase of this pest has led to the intensive use of insecticides, and consequently the selection of resistant populations, which makes it difficult to control them (Diez-Rodriguez and Omoto, 2001). Hence, the use of insecticidal plant extracts could be a promising control tool. In the last decade'sneem tree (*Azadirachta indica* L.) has been used in many parts of the world to control pests in crops (Gutiérrez, 2010). Leaf extracts from neem have shown low efficacy in pest control which limits their widespread use (Osuma, 2005). For this reason, it is necessary to evaluate new alternatives to improve pest control from plant extracts and thereby reduce the use of commercial synthetic inputs that increase production costs, as well as being harmful to human health and the environment (Bahena, 2003).

A potential option to increase the effectiveness of neem leaves as a pesticide will be to combine it with other tree species used for the same purpose. For example, in the Yucatan peninsula of Mexico, there existrich diversity of multi-purpose tree species such as cocoite (*Gliricidia sepium* (Jacq.) Kunth ex Walp.) and huaxin (*Leucaena leucocephala* (Lam.) de Wit) that could be used to control various pests since they are rich in various secondary metabolites like tannins, saponins, phenolic compounds, terpenoids, sulfur, steroids and alkaloids for their defense mechanisms (Cortez, 2002).

In spite of the above, the positive and negative effect that could have the combination of the vegetal extracts is not known in comparisonto pure neem extract for the control of pests such as *S. frugiperda*, principal pest of maize cultivation in the state of Quintana Roo, Mexico. The purpose of the present study was to evaluate the efficacy of *A. indica*, *G. sepium* and *L. leucocephala*, pure and combined plant extracts, and nutritional alterations of *S. frugiperda* under laboratory conditions.

Material and Methods

The study was carried out in the multipurpose laboratory of the Instituto Tecnológico de la Zona Maya, located on the road Chetumal-Escárcega km. 21.5, the ejido Juan Sarabia, Othón P. Blanco, Quintana Roo, Mexico, with geographical coordinates 18°30"58' N 88° 29"19'W.

Fall armywormswith no history of insecticide application were collected in the community of Morocoy (Fig. 1), in the municipality of Othón P. Blanco, Quintana Roo.Subsequently, they were deposited in Petri dishes (15 worms per Petri dish) and fed with fresh and tender leaves of corn until transferred to the biological control laboratory for reproduction (Fig. 1). The worms were individually placed in Petri dishes and feed daily with tender leaves of corn germinated to the pupa stage. For reproductionat adult stages, the technique described by Chacon *et al*. (2009) was used. For the bioassays, larvae of first stage of the foot of breeding maintained in laboratory were used; 56 larvae (8 larvae for each treatment) were selected, with similar weights (0.3 ± 0.05 g), and the same larval stage, which were individually placed in Petri dishes and fed in the same manner previously described.

Fig. 1: *Spodoptera frugiperda*, principal pest of maize cultivation in the state of Quintana Roo, México; **A** = Maice (*Zea Mays* L); **B** = Damage to the corn stalk by the fall armyworm; **C** = *Spodoptera frugiperda*; **D** = Detail of damage to the corn stalk by the fall armyworm

Fig. 2: Plantsused for control of *S. frugiperda*, principal pest of maize cultivation in the state of Quintana Roo, México; **A** =*Azadiracta indica*; **B** =*Gliricidia sepium;* **C** =*Leucaena leucocephala;***D** =*Zea mays*

Two collections of fresh leaves of *A. indica*, *G. sepium*and *L. leucocephala* (Fig. 2A-C) were made in the field area of the ITZM; One in the rainy season (October-December) and another in the dry season (March-May). During each season, 250 g of fresh leaves per 1 liter of water, according to the method of Osuma (2005). Six, three combined aqueous extracts, three pure, and a control (purified water only) were performed. The fresh leaves of the three species were separated, weighed and crushed in a liter of water and the final product was stored for 24 h in plastic containers and subsequently filtered to obtain the aqueous phase.

For the bioassays, the efficacy of the plant extracts on *S. frugiperda* was evaluated. For this purpose, 56 larvae of the same weight and larval status were randomly selected and were individually deposited in Petri dishes and fed with 1.5 g of tender maize grain and 1.5 g of tender maize leaves (3 g per experimental unit) twice during the bioassay (Fig. 2D). Each treatment had 10 replicates in a completely randomized experimental design. The aqueous extracts were applied by direct spray of 3 ml of each extract in each experimental unit. Two applications of extract, day 1 and 4 of the study were performed. The mortality of the worms was recorded daily for a period of 8 days. After 48 h, each left over food was removed and weighed to estimate the consumption of each individual. The final weight of *S. frugiperda* was also recorded at the time of death. The efficacy of the six treatments was calculated using the formula by Alvarez *et al*. (2007) described below:

$$Efficacy(\%) = \frac{A-B}{A} \times 100$$

Where: A is the control group and B is the treated group.

On the other hand, the nutritional alterations of *S. frugiperda* were calculated according to the equations reported by Álvarez *et al*. (2007):

$$Consumptionindex = D/Bt$$

$$Growthrate = (A - B)/Bt$$

$$Indexofuseoffood = D / At$$

Where: A = final larval weight, B = initial larval weight, t = experiment period, and D = food eaten during the experiment period.

Analysis of data

One-way analysis of variance was applied to determine the effect of different aqueous extracts on efficacy and nutritional alterations. When differences were found, a Tukey test was applied with the statistical package Sigmaplot® version 11.0 for Windows®.

Results and discussion

The final weight showed significant differences between the different treatments, with the lowest weight of the larvae treated with the extracts in comparison with the control. Also, food consumption during the period showed significant differences where the lowest food intake was for the extracts of Ai, Ll, Ai + Ll, Ai + Gs and Ai + Ll + Gs, and the highest for the control (Table 1). This is consistent with that reported by Alvarez *et al.* (2007) who indicate that the use of plant extracts based on Annonaceous acetogenins shows anti-food effects, consequently the growth of *S. frugiperda* larvae and its food consumption was lower compared to the control treatment.

Table 1: Final weight of *Spodoptera frugiperda* Larvae and it´s food consumption after the application of different plant extracts made with foliage of three tree species

Treatments	Final weight (g)	Consumption (g)
Ai	0.18 b	0.66 c
Ll	0.20 b	0.77 c
Gs	0.24 b	1.28 b
Ai + Ll	0.23 b	0.53 c
Ai + Gs	0.15 b	0.56 c
Ai + Ll + Gs	0.17 b	0.38 c
Control	0.48 a	3.59 a
Standard error	0.05	0.24
P-value	0.03	0.01

Ai = *Azadiracta indica*; Ll = *Leucaena leucocephala;* Gs = *Gliricidia sepium*; Means followed by different literals indicate that they are different in each column according to the Tukey test

Table 2: Average nutritional alterations in *Spodoptera frugiperda* Larvae after the application of different plant extracts made with foliage of three tree species.

Treatments	Consumption index	Growth index	Food utilization index
Ai	0.75 c	0.04 b	0.99 c
Ll	0.48 d	-0.04 b	0.56 d
Gs	1.27 b	-0.03 b	1.63 b
Ai + Ll	0.24 e	-0.05 bc	0.36 e
Ai + Gs	0.28 e	-0.06 c	0.48 de
Ai + Ll + Gs	0.30 e	-0.05 bc	0.53 d
Control	3.71 a	0.24 a	4.22 a
Standard error	0.21	0.02	0.18
P-value	0.01	0.01	0.01

Ai = *Azadiracta indica*; Ll = *Leucaena leucocephala;* Gs = *Gliricidia sepium*; Means followed by different literals indicate that they are different in each column according to the Tukey test

In relation to the nutritional alterations, it was possible to indicate that the groups administered pure and combined vegetable extracts had a lower consumption index (P <0.01), lower growth index (P <0.01) and lower food utilization index (P <0.01) compared to the control.Studies related to nutritional alterations caused by plant extracts are currently scarce (Table 2). Álvarez *et al*. (2007) evaluated the toxic effects of acetones on the seeds of the Anonaceae species on *S. frugiperda* and reported a clear decrease in the growth rate of the larvae. However, the ketones did not significantly affect the consumption index and the rate of utilization of the food by *S. frugiperda* larvae. This is contrary to our current findings, where it was observed that the aqueous extracts of *A.indica, L. leucocephala* and *G. sepium*, pure and combined showed a significant effect on the growth index, consumption index and food utilization rate in *S. frugiperda* larvae.

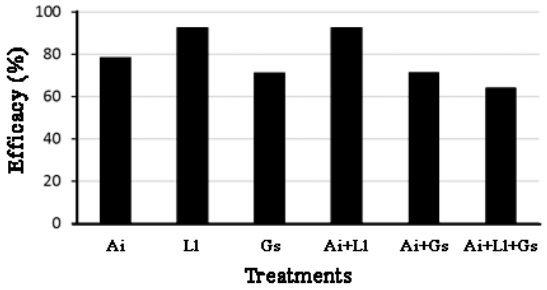

Fig. 3: Percentage of average efficiency of plant extracts made with foliage of three tree species for the control of *S. frugiperda* under *in vitro* conditions; Ai = *Azadiracta indica*; Ll = *Leucaena leucocephala*; Gs = *Gliricidia sepium*

The Fig. 3 presents the efficacy of the *S. frugiperda* exposed to different aqueous extracts made with fresh leaves of three tree species. The results showed that the highest average efficiency was for *L. leucocephala* pure extract (92.85%) and mixed *A. indica* with *L. leucocephala* (92.85%), compared to the other plant extracts (P = 0.032) (Fig. 2). In the present study, it was observed that the pure extract of *L. leucocephala* and combined with *A. indica* showed high efficiencies compared to the other extracts. This may be due to the fact that this species shows high concentrations of condensed tannins, total phenols or glycosides of phenols (Bryant *et al*., 1995) compared to the other species studied. This agrees with Chaves *et al*. (2001) who reported that earlier reporting that the extract of *Cistus landanifer* has a high concentration of phenols; and that factors such as radiation, UV rays and water stress increased the concentration of this metabolite during the spring. On the other hand, it is important to emphasize that the present study is one of the first reports in Mexico using *L. leucocephala* extracts for the control of *S. frugiperda* larvae. This legume has wide distribution across tropical Mexico and well known for their forage and nutritional properties (Casanova-Lugo *et al*., 2014); and has not been reported earlier in maize pest control.

Conclusion

The use of pure and combined plant extracts adversely affects the growth of *S. frugiperda* larvae, however, the extract that *G. sepium* had less effect on the food consumption of the larvae than the other extracts. On the other hand, the combined extracts of *A. indica* with *L. leucocephala* and *G. sepium* and the pure extract of *L. leucocephala* influenced the Consumption index, growth index and food utilization index. Despite the above, the best efficacy values were for extracts of *A. indica* with *L. leucocephala* and *L. leucocephala*.

Therefore, they represent a good organic alternative for the control of *S. frugiperda* in the south of Quintana Roo.

References

Alvarez CO, Neske A, Popich S & Bardon A 2007. Toxic effects of annonaceous acetogenins from Annona cherimolia (Magnoliales: Annonaceae) on *Spodoptera frugiperda* (lepidóptera: Noctuidae). *J. Pest Sci.*, 80: 63–67.

Bahena J, Arredondo C, Sánchez M & Miranda S 2003. Extractos vegetales y bioplaguicida, alternativas para el combate del gusano cogollero del maíz *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). *Entomol. Mex.*, 2: 366–372.

Bryant J & Julkenen TR 1995. Ontogenic development of chemical defense by seedling resing birch: energy cost of defense production. *J. Chem. Ecol*., 21: 883–896.

Casanova-Lugo F, Petit-Aldana J, Solorio-Sánchez F, Parsons D & Ramírez-Avilés L 2014. Forage yield and quality of *Leucaena leucocephala* and *Guazuma ulmifolia* in mixed and pure fodder banks systems in Yucatan, Mexico". *Agrofores Syst.*, 88(1): 29–39.

Chacón C, Garita R, Vlaglio C & Christopher C 2009. Desarrollo de una metodología de crianza en laboratorio del gusano cogollero del maíz *Spodoptera frugiperda* (Smith), (Lepidoptera: Noctuidae) como posible hospedante de insectos biocontroladores de interés Agrícola. *Tecnología en Marcha*, 22(4):28–37.

Chaves N, Sosa T & Escudero JC 2001. Plant growth inhibiting flavonoids in exudate of *Cistus ladanefer* and in associated soils. *J. Chem. Ecol*., 27: 623–631.

Cortez M 2002. Evaluación de extractos vegetales para el control del gusano cogollero *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) en maíz. Informe técnico. INIFAP.

Diez-Rodríguez G & Omoto C 2001. Herança da resistência de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) a lambda-cialotrina. *Neotrop Entomol*., 30: 311–316.

Gutiérrez G, Sánchez E, Pérez D, Carballo C, Berguinson D & Guilera P 2010. Efecto del nim en el daño ocasionado por el gusano cogollero *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) en tres variables agroquímicas de maíz resistente y susceptible. *Acta Zool. Mex*., 26(1): 1–16.

Moreno LS & González SL 2011. Efecto antifúngico de extractos de gobernadora (*Larrea tridenta*ta L.) sobre la inhibición in vitro de Aspergillus *flavus* y *Penicillium* sp. *Polibotánica*, 32: 193–205.

Osuma E 2005. Uso de Neem para la elaboración artesanal de bioplaguicidas. INIFAP. 13-14.

SIAP [Servicio de Información Agroalimentaria y Pesquera] 2014. Estadísticas de Producción para el Año Agrícola 2012. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. www.siap.gob.mx/index (consulta, 22 de marzo 2016).