

INSECTICIDAL ACTIVITY OF *TAGETES ERECTA* EXTRACTS ON *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The bioinsecticidal activity of organic extracts of *Tagetes erecta* L. (Asteraceae) was evaluated on neonate larvae of *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) a major maize pest in the world. The acetone leaf extract (500 ppm) of *T. erecta* induced an antifeedant effect, causing a 50% reduction of larval weight in comparison with the control. Larval weights were drastically reduced at 7 d, but even more so at 14 d, when *T. erecta* extracts also caused substantial mortality. Three leaf extracts of *T. erecta* caused high larval mortality, with hexane (48%), acetone (60%) and ethanol (72%). Further *T. erecta* leaf extracts caused pupal mortalities of 40-80%. The use of such plant extracts can be proposed as bioinsecticides to control *S. frugiperda*, in a more environment-friendly manner.

Key Words: bioinsecticide, organic extracts

RESUMEN

Se evaluó la actividad bioinsecticida de extractos orgánicos de *Tagetes erecta* L. (Asteraceae) sobre larvas neonatas de *Spodoptera frugiperda* J. E. Smith. (Lepidoptera: Noctuidae) plaga más importante del maíz en el mundo. El extracto acetónico de hojas (500 ppm) de *T. erecta* mostró un efecto antialimentario respecto a los extractos florales de esta especie ocasionando una reducción del 50% del peso de las larvas en comparación al testigo. La información obtenida de los pesos de las larvas a los siete días se corroboró con los resultados obtenidos a los 14 días, los tres extractos de hoja de *T. erecta* provocaron alta mortalidad larval, con hexano (48%), acetona (60%) y hoja etanol, un 72%. Los extractos de *T. erecta* de hojas presentaron una actividad tóxica causando una mortalidad en pupas del 40 al 80%. El uso de extractos de plantas puede ser propuesto como un método para el control de *S. frugiperda*, de una manera respetuosa del medio ambiente.

Grains with the greatest market demand are corn, wheat, rice, and sorghum (Pandya-Lorch et al. 2001; Álvarez 2006; Eyherabide 2006; Geard 2006). The productivity of corn is constantly affected by biotic and abiotic conditions. Insect pests are of great concern to corn farmers, because they jeopardize grain production (Alonso & Martínez 1990; Fernández 1998). Consequently, farmers employ synthetic chemical insecticides to control or eliminate pests, often without adequately considering the manifold negative potential consequences. The latter may include ecological disasters, poor crop quality, and negative effects on the health of fauna and humans (Ramón 2007). This set of problems has given rise to the use of plants as a potential source of the safest pesticides for

the environment and for human health (Ottaway 2001; Mansaray 2000).

Thus, in recent years, research on plant bioinsecticides for controlling the fall armyworm (*Spodoptera frugiperda*) has been renewed, because this pest is one of the most important in causing economically significant damage to a great variety of agricultural production systems (rice, cotton, oatmeal, barley, potato, tomato, tobacco, garden vegetables, among others, although it exhibits the greatest preference for corn) (Andrew 1998). The *Tagetes* genus (Asterales: Asteraceae) is an alternative candidate for the control of pests and diseases, because it contains secondary metabolites. As sources of pesticides, *Tagetes lucida*, Cav., *Tagetes filifolia* Lag, *Tagetes foetidissima*

sima DC, and *Tagetes coronopifolia* Willd. are promising (Serrato et al. 2007). Results of trials nurseries and in agricultural fields indicate that the employment of *Tagetes* species in agricultural production systems have the potential to displace much of the current use synthetic pesticidal products (Serrato et al. 2007).

Tagetes species possess the following secondary metabolites in their flowers, seeds, and roots: alilanisol, anetol, limonene, methyl eugenol, and β -karyophyllene that are have toxic to insects, mites, nematodes, bacteria, fungi, and viruses. Such compounds have been reported to be present in *Tagetes* essential oil, and they belong to certain groups of hydrocarbons, alcohols, ethers, aldehydes, ketones, esters, carotenoids, flavonoids and thiophenes, (Camarillo et al. 2007).

Cubillo et al. (1999) evaluated repellency and toxicity to *Bemisia tabaci* Gennadius of commercial insecticides and plant extracts of the *Tagetes* genus. They reported that the ethanolic extract of *T. filifolia* roots inhibited oviposition 60% at 48 h; repelled 55% at 24 h and killed 49% at 48 h. Also Vidal et al. (2009) evaluated the toxicity of *Tagetes patula* ethanolic leaf extracts on *Aedes aegypti* L. 4th instar larvae and pupae; they reported 92 and 77% mortality of larvae and pupae, respectively, caused by 153.6 mg/L of the extract at 48 h.

The objective of the present study was to bioassay the effects of organic extracts of *Tagetes erecta* leaves and flowers on *S. frugiperda* larval development.

MATERIALS AND METHODS

Acquisition and Preparation of *Tagetes erecta* Plant Material

Tagetes erecta was gathered in the flowering season (Oct and Nov 2009) in the Tepoztlán Municipality, Morelos State, Mexico, and a sample was deposited at the Autonomous University of the State of Morelos Herbarium (HUMO) with registry number 27192. Later, the collected plant material was dried at room temperature in a dark room, and the leaves and flowers were separated in order for these to be triturated with the help of a pulvex plastic hand mill Model 95 (Molinos Pulvex, S.A. de C.V, México) to obtain pulverized samples of each of the plant's parts.

Insects - *Spodoptera frugiperda*

The fall armyworm larvae were provided by the Entomology Department, Biotic Products Development Center of the National Polytechnic Institute (CEPROBI-IPN) laboratory nursery. The larvae were maintained in a Precision model 818 incubation chamber at a temperature of 27 ± 1 °C, with 60-70% RH at 12:12 h L:D, and reared on

a meridic diet (Burton & Perkins 1987). Second-generation (F_2) larvae were used for all experiments, and 100 neonate larvae were used for each treatment.

Meridic Diet for Rearing *Spodoptera frugiperda*

We prepared the fall armyworm meridic diet (Burton & Perkins 1987) for mass rearing and for conducting the bioevaluations with the following ingredients: Peruvian bean (*Phaseolus vulgaris* L.) (11.27%), wheat germ (5.16%), beer leavening (3.28%), ascorbic acid (0.32%), sorbic acid (0.10%), methyl parahydroxybenzoate (0.20%), 0.94% of formaldehyde at 10%, agar (1.40%), water for the beans (43.53%), and water for the agar (33.80%).

Crude Extract Preparation of *Tagetes* Flowers and Leaves

For preparation of the crude extracts, the flower and leaf material (500 g of each), dry pulverized separately, were placed in a 500 mL round, flat-bottomed flask, into which one of the following solvents was added until the material was covered completely: *n*-hexane (Fermont), or acetone (Mallinckrodt), or ethanol (J. T. Baker). Maceration was performed for 3 d.

Once maceration had been carried out, the solid material was separated from the liquid, and the solvent was eliminated by reduced-pressure distillation (21 torr) in a Buchi 205 rota-evaporator with the purpose of obtaining the 2 plant crude extracts (flowers and leaves). The solvents were removed by evaporation in the process of obtaining extracts. The extracts stored in amber glass bottles to avoid photolysis and stored at -20 °C in a freezer, until they were used in the bioassays.

Bioassays

Each of the various crude extracts of *T. erecta* flowers and leaves (*n*-hexane, acetone and ethanol) was incorporated into the above meridic diet at final concentrations of 500 ppm and the effects of these preparations on the development and survival of *S. frugiperda* larvae were bioassayed. Each of the control diets was prepared with 1mL either of *n*-hexane, acetone or ethanol. Diet ingredients and the concentrate extracts were mixed following the protocol of Franco et al (2006), and the prepared mixture was dispensed into cylindrical plastic containers (3 cm high \times 3.5 cm diam) with 15 mL per container. Once the diet had cooled and solidified, 1 neonate larva was placed in each container with the aid of a fine camel hair brush. This process was replicated 2 times with 50 neonate larvae per replication. The containers were randomly arranged in a climactic chamber under the same conditions as used for rearing the laboratory colony of *S. frugiperda*.

The experimental design was completely randomized with 7 treatments and 2 replications ($n = 100$ larvae). Response variables were as follows: larval weights at 7 and 14 d, larval development, larval mortality, pupal viability and adult emergence. Mortality was calculated by means of Abbot's formula (Abbot 1925). The statistical analysis carried out was the analysis of variance (ANOVA) and mean comparison, i.e., mean \pm standard deviation (MSD) ($P = 0.5$).

RESULTS AND DISCUSSION

Effect of *Tagetes* Leaf Extracts on Weights of *S. frugiperda* Larvae

In Table 1 are shown the mean weights of larvae treated with the various *T. erecta* flowers and leaf extracts at 7 d. Statistically significant differences occurred between the mean weights of larvae in the untreated control (0.0186 g) and those of larvae treated with leaf extracts with n-hexane, acetone and ethanol, which had mean weights of 0.0033, 0.0019, and 0.0014 g, respectively. Larvae treated with leaf extracts had significantly lower weights than those fed the corresponding floral extracts.

At 14 d, the mean weight of control larvae was 0.3140 g, while the mean weights of larvae treated with the leaf extracts of n-hexane, acetone and ethanol were merely 0.1669, 0.1844, and 0.0746 g, respectively. Thus the profound effect of *T. erecta* leaf extracts on retarding gains in larval weight first demonstrated at 7d was corroborated. These data suggest that the leaf extracts may inhibit feeding, and hence they may be antifeedants. At 14 d the ethanol leaf extract clearly had the most drastic effect. Probably the toxic components in the leaf extracts include phagodeterrents.

Argueta & Vazquez (1994) reported that the leaves of *T. erecta* contain essential oils, which have been identified as geraniol, limonene, linalol and its acetate, menthol, ocimene, beta-phel-

landrene, dipentene, alpha and beta-pinene and tagetona. According Coll & Esquivel (2009), these compounds have insect antifeedant activities.

These results are in agreement with those obtained by Picman (1986), who performed a review on the biological activity of the sesquiterpenes, and concluded that some sesquiterpenes may possess feeding inhibitory activity. There are other studies on this effect carried out on lepidopterans. Blaney et al. (1990) concluded that feeding inhibition is caused by azadirachtin and limonoids. They conducted a study on the effects of azirdiractin and related compounds on feeding by 4 noctuid species (*Spodoptera littoralis* Boisduval, *S. frugiperda*, *Heliothis virescens* F., and *H. armigera* Hübner), and reported severe antifeedant activity against *S. littoralis* and, to a lesser degree, against *S. frugiperda*, *H. virescens*, and *H. armigera*.

Caballero (2004) evaluated the antifeedant activity caused by 3 groups of terpenoids (neoclerodane diterpenes, neo-clerodane sesquiterpenes, and limonoids) against *Spodoptera exigua* Hübner and the coleopteran, *Leptinotarsa decemlineata* Say, with results that suggested to us that we would expect to identify an antifeedant effect caused by terpenes against *S. frugiperda*, because it is congeneric with *S. exigua*. The latter is possible because both species share morphological, phylogenetic, and ecological characters, and they cope with similar secondary metabolites.

Effects of *Tagetes* Flower and Leaf Extracts on Larval mortality

In Table 2 are shown the observed percentages of mortality of *S. frugiperda* larvae treated with the *T. erecta* flower and leaf extracts made with n-hexane, acetone and ethanol. In the control (no extract) *T. erecta*, mortality was 0%, the flower extracts cause low mortality, while the leaf extracts caused substantial mortality (Table 2). According to the criterion proposed by Silva et al. (2003), plants and/or extracts with promise as bioinsec-

TABLE 1. EFFECT OF EXTRACTS OF *TAGETES ERECTA* FLOWERS AND LEAVES INCORPORATED INTO MERIDIC DIET ON THE WEIGHTS OF *SPODOPTERA FRUGIPERDA* LARVAE AT 7 AND 14 D. ALL EXTRACTS HAD BEEN INCORPORATED INTO THE MERIDIC DIET AT 500 PPM.

Treatment (<i>Tagetes erecta</i> extract)	<i>Spodoptera frugiperda</i> larval weight (g) mean \pm SD	
	7 days	14 days
Control	0.0186 \pm 0.0079 a*	0.3140 \pm 0.1161 a
n-hexane flower	0.0124 \pm 0.0053 b	0.2290 \pm 0.856 bc
ethanol flower	0.0098 \pm 0.0047 c	0.2448 \pm 0.0954 bc
acetone flower	0.0094 \pm 0.0045 c	0.2200 \pm 0.1146 bcd
n-hexane leaf	0.0033 \pm 0.0030 de	0.1669 \pm 0.1578 de
acetone leaf	0.0019 \pm 0.0012 def	0.1844 \pm 0.0781 cde
ethanol leaf	0.0014 \pm 0.0010 ef	0.0746 \pm 0.0089 fg

*ANOVA, Standard deviation (SD), and MSD ($P = 0.5$) tests were carried out.

Means followed by the same letters in each column are not significantly different.

TABLE 2. EFFECT OF EXTRACTS OF *TAGETES ERECTA* FLOWERS AND LEAVES INCORPORATED INTO MERIDIC DIET ON THE MORTALITY OF *SPODOPTERA FRUGIPERDA* LARVAE. ALL EXTRACTS HAD BEEN INCORPORATED INTO THE MERIDIC DIET AT 500 PPM.

Treatment (<i>Tagetes erecta</i> extract)	% Mortality of <i>Spodoptera frugiperda</i> larvae
Control	0*
n-hexane flower	12
ethanol flower	14
acetone flower	24
n-hexane leaf	48
acetone leaf	60
ethanol leaf	72

*Number of larvae per treatment was 100.

ticides are those that cause a mortality > 40%. According to this criterion, *T. erecta* leaf extracts with hexane, acetone, or ethanol all were bioinsecticidal, because they produced mortalities of 48, 60, and 72%, respectively. Therefore, *T. erecta* has the toxic potential to be considered for as a source of bioinsecticides to control the fall armyworm.

Effects of *Tagetes* Extracts on Duration of Larval Development

Table 3 shows the effects of the 6 phyto-extracts on the duration of *S. frugiperda* larval development. The durations of the larval stadia in the acetone flower and acetone leaf extract treatments did not differ significantly from that of the control (18.2 d \pm 0.169e). Duration of the larval stadia was prolonged the most by the n-hexane flower extract treatment (41 d \pm 2.407a) followed by the ethanol leaf extract (33.9 d \pm 9.815b) treatments. The apparent slight prolongations of duration of the larval stadia in the ethanol flower, acetone flower and acetone leaf extract treatments did not

TABLE 3. EFFECT OF EXTRACTS OF *TAGETES ERECTA* FLOWERS AND LEAVES INCORPORATED INTO MERIDIC DIET ON THE DURATION OF DEVELOPMENT OF *SPODOPTERA FRUGIPERDA* LARVAE. ALL EXTRACTS HAD BEEN INCORPORATED INTO THE MERIDIC DIET AT 500 PPM.

Treatment (<i>Tagetes erecta</i> extract)	Mean duration of larval stadia (days) prior to pupation
Control	18.2 \pm 0.169 e*
n-hexane flower	41.0 \pm 2.407 a
ethanol flower	21.3 \pm 2.901 cd
acetone flower	20.1 \pm 2.198 cde
hexane leaf	22.4 \pm 7.684 c
acetone leaf	18.9 \pm 1.630 de
ethanol leaf	33.9 \pm 9.815 b

*ANOVA, Standard deviation (SD), and MSD ($P = 0.5$) tests were carried out. Means followed by the same letters in each column are not significantly different.

differ significantly from each other. These results suggest that the n-hexane flower and ethanol leaf extracts possess metabolites with a phytoecdysteroid effect (Alonso 1998).

Effects of *Tagetes* Flower and Leaf Extracts on % Mortality of Pupae

Data in Table 4 demonstrate that the *T. erecta* flower extracts were not highly toxic to *S. frugiperda* pupae with the ethanol flower extract causing no mortality and the n-hexane flower and acetone flower extracts causing only 12% and 11% mortality, respectively. On the other hand, the various *T. erecta* leaf extracts caused pupal mortalities in the range of 57 to 80%; indeed the acetone leaf extract caused the greatest mortality. In each treatment the various *T. erecta* leaf extracts caused combined mortalities of larvae and pupae in the range of 80 to 97% (Table 4).

CONCLUSIONS

This study showed that leaf extracts of *T. erecta* are toxic to *S. frugiperda* larvae with significant toxic effects extending into the pupal stage. The ethanol leaf extract caused the highest % mortality. Moreover, *T. erecta* leaf extracts caused additional high mortality in the pupal stage ranging from 62-80%. These extracts caused these substantial mortalities through antifeedant and insecticidal actions.

The durations of the larval stadia was increased from 18 d in the control to 34 d and 41 d in the n-hexane and ethanol floral extract treatments, respectively.

Considering the results obtained in this study together with the data already reported in the scientific literature, it is clear that species of the Asteraceae contain several chemical classes of bioactive compounds. Certain terpenes have very great antifeedant, phagodeterrent, and toxic effects on herbivorous insects. We will continue our phytochemical studies to determine which terpenes present in *T. erecta* are the most bioactive. The ethanol and acetone leaf extracts of *T. erecta* appear to contain highly bioactive compounds relevant to the control of *S. frugiperda*.

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TABLE 4. EFFECT OF EXTRACTS OF *TAGETES ERECTA* FLOWERS AND LEAVES INCORPORATED INTO MERIDIC DIET ON THE MORTALITY OF *SPODOPTERA FRUGIPERDA* PUPAE AND THE COMBINED MORTALITIES OF LARVAE AND PUPAE. ALL EXTRACTS HAD BEEN INCORPORATED INTO THE MERIDIC DIET AT 500 PPM.

Treatment (<i>Tagetes erecta</i> extract)	% Mortality of <i>Spodoptera frugiperda</i> pupae	Combined % mortalities of <i>S. frugiperda</i> larvae and pupae
Control	0*	0
n-hexane flower	12	24
ethanol flower	0	14
acetone flower	11	32
n-hexane leaf	62	80
acetone leaf	80	92
ethanol leaf	57	88

*Pupae were the survivors of the larvae used in these bioassays.

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