Effects of Ethanolic Extracts of Argemone ochroleuca (Papaveraceae) on the Food Consumption and Development of Spodoptera frugiperda (Lepidoptera: Noctuidae)

Authors: Ana M. Martínez, Aldo J. Aguado-Pedraza, Elisa Viñuela, Christian L. Rodríguez-Enríquez, Philippe Lobit, et. al.

Source: Florida Entomologist, 100(2) : 339-345
Published By: Florida Entomological Society
URL: https://doi.org/10.1653/024.100.0232
Effects of ethanolic extracts of *Argemone ochroleuca* (Papaveraceae) on the food consumption and development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Ana M. Martínez¹, Aldo J. Aguado-Pedraza², Elisa Viñuela³, Christian L. Rodríguez-Enríquez¹, Philippe Lobit¹, Benjamín Gómez¹, and Samuel Pineda¹,*

Abstract

The effects of 2 concentrations (15 and 30%) of ethanolic *Argemone ochroleuca* Sweet (Papaveraceae) extracts were determined on the feeding behavior and development of 3rd instar larvae of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) through their continuous ingestion of treated maize leaves for 48 h. The extracts were obtained from dried whole plants including stems, leaves, and flowers. Armyworm food consumption was slightly reduced (by 13 to 14%) at both concentrations compared with the controls. Larval growth was significantly affected only at the highest concentration, with a 23% reduction in larval weight after 48 h of feeding on the treated leaves and a 38% reduction after another 48 h of feeding on an untreated artificial diet. By contrast, the duration of the larval and pupal stages was prolonged by 43% (from 13.5 to 19.4 d) and by 8% (from 2.1 to 2.2 d), respectively. Total larval mortality was also higher at the highest concentration (31%) than in controls (10%). During the pupal stage, neither the number of pupae that formed nor their mortality or weight was significantly affected by the treatments. However, both of the bioassay concentrations significantly lengthened the duration of the pupal stage in males, whereas in females, only the higher concentration did. In addition, no effect was observed on the adult emergence, sex ratio, and fecundity. In summary, the primary effect of ethanolic *A. ochroleuca* extracts appears to be a reduction in feeding, which simultaneously slows larval growth and increases mortality.

Key Words: botanical insecticide; fall armyworm; effect on development; feeding behavior

Resumen

En este estudio se determinó, a través de la ingestión continua de hojas tratadas de maíz durante 48 h, el efecto de dos concentraciones (15% y 30%) de extractos etanólicos de *Argemone ochroleuca* Sweet (Papaveraceae) sobre el comportamiento de alimentación y desarrollo de larvas de tercer instar del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). Los extractos se obtuvieron de plantas completas secas incluyendo tallos, hojas y flores. El consumo de alimento se redujo ligeramente (en 13 a 14%) en ambas concentraciones comparado con el testigo. El crecimiento fue afectado significativamente solamente en la más alta concentración, con una reducción de 23% del peso larval después de 48 h de alimentación sobre las hojas tratadas y un 38% de reducción después de otras 48 h de alimentación sobre dieta artificial no tratada. En contraste, la duración de los estados de larva y pupa se incrementó en 43% (desde 13.5 a 19.4 d) y 8% (desde 2.1 a 2.2 d), respectivamente. La mortalidad larvaria también fue más alta en la concentración más alta (31%) que en el testigo (10%). Durante el estado de pupa, ni el número de pupas formados ni su mortalidad o peso fue afectado significativamente por los tratamientos. Sin embargo, ambas concentraciones ensayadas prolongaron significativamente la duración del estado de pupas en machos mientras que en hembras este efecto solamente se observó en la concentración más alta. También, ningún efecto se observó en la emergencia de adultos, proporción de sexos y fecundidad. Se concluye que el principal efecto de los extractos etanólicos de *A. ochroleuca* son la reducción en la alimentación, lo cual, al mismo tiempo, prolonga el crecimiento larval e incrementa la mortalidad.

Palabras Clave: insecticidas botánicos; gusano cogollero; efectos en el desarrollo; comportamiento de alimentación

The widespread use of synthetic insecticides for controlling insect pests has led to many detrimental effects such as the development of pest resistance, environmental pollution, interference with biological control, undesirable effects on workers, the presence of residues in food if safety periods are not observed, and a loss of vital plant pollinators (Pimentel & Peshin 2014). For these reasons, scientists and growers are continuously seeking new tools that are effective against the target pest, are compatible with integrated pest management prac-

¹Universidad Michoacana de San Nicolás de Hidalgo, Instituto de Investigaciones Agropecuarias y Forestales, Km. 9.5 Carretera Morelia-Zinápécuaro, 58880 Tarimbaro, Michoacán, Mexico; E-mail: amabel_66@hotmail.com (A. M. M.), saap_3k15@hotmail.com (A. J. A.-P.), chrisluroen@gmail.com (C. L. R.-E.), plobit@gmail.com (P. L.), spineda_us@yahoo.com (S. P.)

²Instituto Tecnológico del Valle de Morelia, Km. 6.5 Carretera Morelia-Salamanca, 58100 Los Ángeles, Morelia, Michoacán, Mexico

³Protección de Cultivos, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, E-28040 Madrid, Spain; E-mail: elisa.vinuela@upm.es (E. V.)

¹Universidad Michoacana de San Nicolás de Hidalgo, Facultad de Medicina Veterinaria y Zootecnia, Km. 9.5 Carretera Morelia-Zinápécuaro, 58880 Tarimbaro, Michoacán, Mexico; E-mail: roschberith@gmail.com (B. G.)

*Corresponding author; E-mail: spineda_us@yahoo.com (S. P.)
icates (Copping & Menn 2000; Garcia et al. 2006), and have a favorable ecotoxological profile including short persistence in the environment (Isman 2006; Sentinel-Nathan 2015).

Botanicals have a long history of use. The use of plant derivatives that can act as antifeedants, repellents, and toxicants extends back at least 2 millennia in some countries (Egypt, China, Greece, and India) and more than 150 yr in Europe and North America (El-Wakeel 2013). However, besides pyrethrum, neem, and some plant essential oils, only a few plant-derived substances are available as commercial products in the world (Sola et al. 2014), even though these products are key tools in organic agriculture (DOUE 2016). At present, crude plant materials or extracts and a few commercial formulations produced by farmers and small-scale industries are applied to crops in order to target various pests in Africa, Asia, and Latin American countries (Dubey et al. 2011; Isman & Paluch 2011). In Mexico, botanical pesticides that were extracted from plants of various families [e.g., Allium sativum L. (Amaryllidaceae), Cinnamomum verum J. S. Presl. (Lauraceae), Capsicum frutescens L. (Solanaceae), Larrea tridentata (Moq. and Sesse ex DC.) Coville (Zygophyllaceae), Azadirachta indica A. Juss (Meliaceae), and Trichilia havanensis Jacq. (Meliaceae)] were evaluated for their control of lepidopteran, hemipteran, thysanopteran, and mite pests in horticultural and berry crops (López-Olguín et al. 1999, 2000; A. J. Aguado-Pedraza, Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, personal communication). In recent years, the interest in botanical pesticides has resurfaced globally due to the risk associated with synthetics, and the voluminous scientific literature describing their effects includes several books (see review articles by Isman 2006; El-Wakeel 2013). Although botanical pesticides make up only approximately 1% of the global insecticide market (Isman & Paluch 2011), they are essential in organic agriculture. These compounds have been reported to be safer for beneficial organisms than conventional products (Croft 1990; Hasseeb et al. 2004).

Plants and herbivorous insects have evolved together since the Carboniferous period (Wink 2003; Abdul Majeed & Abidunna 2011). The diversity of plant allelochemicals and their chemical structures is enormous, and to date, more than 100,000 compounds have been isolated, and their roles in preventing insect damage have been elucidated (Koul 2005). In contrast to the mode of action of many synthetic pesticides, only a few of these secondary metabolites target the insect nervous system. Plant allelochemicals act primarily as inhibitors of growth, development, and reproduction; as antifeedants and inhibitors of oviposition; and as repellents (Isman & Paluch 2011). Based on the results of our group, some metabolites from Cestrum parqui (Lamarck) L’Héritier (Solanaceae) and Drimys winteri J. R. Forster & G. Forster (Winteraceae) offer excellent potential as pesticides due to their antifeedant and toxic activity against key pests from various orders (Zapata et al. 2006, 2009, 2010).

Our study was focused on Argemone ochroleuca Sweet (Papaveraceae), a plant that is native to Mexico and known locally as “chicxolte,” which is widely distributed from the southern United States across Mexico to Central and South America, the Caribbean, Australia (Heard & Segura 2012; Brahmachari et al. 2013), India (Sharma et al. 2010; Patel 2013) and Africa (Ibrahem & Ibrahim 2009). This species is an annual prickly herb (growing up to 1 m high) that grows year-round and can be distinguished from other species in the genus by the presence of yellow sap and pale yellow petals (Heard & Segura 2012; Patel 2013). The aerial parts of A. ochroleuca have been used in traditional human medicine for mitigation of some illnesses such as skin diseases (warts and spots), insomnia, nervous diseases, eye infections, coughs, microbial infections, and rage (Rzedowski 1991; Argueta & Cano 1994). Fatty acids (Fletcher et al. 1993), flavonoids (Bhardwaj et al. 1982; Saleh et al. 1987; Chang et al. 2003), and essential oils (Rzedowski 1991) were isolated from A. ochroleuca, and a great variety of alkaloids (berberine, palmatine, protopine, sanguinarine, optinsine, chelerythrine, atropine, dihydrosanguinarine, dihydro-chelerythrine, α-allocryptopine, helierrine, quellantifoline, scoulerine, reticuline, and coptisine) were identified (Israilov et al. 1986; Takken et al. 1993; Espinosa & Sarukhán 1997). It is believed that the high contents of these components protect this species from herbivorous insects. Nevertheless, the insecticidal activity of A. ochroleuca is not well documented. Alkaloids from the Argeome genus possess a wide variety of biological activities, including pharmacological, cytotoxic, antibacterial, antifungal, and antifeedant properties (Schmeller et al. 1997; Chang et al. 2003; Alamri & Moustafa 2010; Brahmachari et al. 2013).

Even though the Argeome genus includes 30 species (Karlsson et al. 2003), most of the information that is available in the literature concerning their medicinal and insecticidal properties has been generated from research on the most abundant species, Argemone mexicana L. (Papaveraceae). Various plant parts of this species (the roots, branches, stems, leaves, flowers, seed extracts, and oils) possess insecticidal, repellent, nematocidal, and bactericidal effects, and they were tested against a wide range of pests of agricultural importance (Prakash & Rao 1997). Crude A. mexicana extracts were demonstrated to have ovicidal and larvicidal activity against lepidopteran, dipteran, coleopteran, and hemipteran pests (Cepero 1994; Zambare et al. 2012; Kangade & Zambare 2013). These extracts have a wide range of sublethal effects including growth delay (Rao & Chitra 2000), reduced fecundity and fertility, molting disorders, morphogenetic defects (Malarvannan et al. 2008; Kangade & Zambare 2013), and repellency (Abdul Majeed & Abidunna 2011).

The only documented case of biological activity of A. ochroleuca on insects is from Bakhshawin & Alqurashi (2010), who found repellent and insecticidal effects of extracts from leaves of this species on the flour beetle Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). Therefore, to help better understand the biological activity of A. ochroleuca, we report the lethal and sublethal effects of ethanolic extracts from a mix of dried stems, leaves, and flowers of this species on 3rd instar larvae of the fall armyworm, Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae), which is the most serious maize pest in Latin America.

Materials and Methods

INSECT REARING

The insects used in these tests came from an S. frugiperda colony that was maintained at the Instituto de Investigaciones Agropecuarias y Forestales (IIAF) of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH) (Tarímbaro, Michoacán, Mexico) and had no history of insecticide exposure. The colony was refreshed annually with the introduction of wild stock (120 to 149 pupae each yr) that were collected from a maize field at El Trébol, within a 9.5 km radius of the town of Morelia, Michoacán, Mexico, to help maintain the genetic variability. The larvae were reared on an artificial diet (Poitout & Bues 1974) and the adults were fed with a 15% honey solution. Brown paper was provided as an oviposition substrate and replaced as needed. Insect rearing and bioassays were performed at 25 ± 2 °C with 75 ± 5% RH and a photoperiod of 16:8 h L:D.

EXTRACT PREPARATION

Six A. ochroleuca flowering plants (approx. 0.80 m in height) were collected in Feb 2013 at the campus of the Universidad Tecnológica de Morelia (19.7494171°N, 101.1688899°W) in Morelia, Michoacán,
Mexico. Specimen identification was conducted through comparisons
with a voucher specimen (No. 46485) that was deposited in the Her-
barium of the Faculty of Biology at the UMSNH. Fresh materials in-
cluding stems, leaves, and flowers were washed with distilled water to
remove any debris and dried in the shade at room temperature for 1
wk. All the dried material was chopped, ground in a porcelain mortar,
and extracted with 500 mL of 96% ethanol (J. T. Baker, Xalostoc, Es-
tado de México, Mexico). This mixture was maintained under labora-
tory conditions at approximately 25 °C for 5 d and gently shaken at 24
h intervals. Finally, it was filtered (paper Whatman® No. 1, Whatman
International Ltd., Maidstone, United Kingdom), and the solvent was
evaporated using a stirring hot plate (Cimarec®, Dubuque, Iowa) at 60
to 65 °C until a concentrated extract was obtained. As reported by Al-
Hayyan (2006), the alkaloids in A. ochroleuca had good extractability in
organic solvents (ethanol, dichloromethane, acetone, petroleum ether,
chloroform, methanol, and ethyl acetate), and thus we decided to use
ethanolic extracts. Dilutions of 15 and 30% (vol/vol) were prepared by
diluting 2.25 mL or 4.5 mL of concentrated extract in water up to 15 mL.

PLANT MATERIAL

Maize seeds (Zea mays L.; Poaceae) of cv. HY-311 were allowed to
germinate for 3 d on damp filter paper and then planted individually in
a polystyrene container (11 cm diameter × 17 cm height) filled with a
mixture of volcanic gravel known locally as "tezontle" and humus-rich
soil (1:1). All of the plants were watered at 3 d intervals and fertilized
once a week with approximately 0.1 g per plant of a commercial gran-
ular fertilizer (NPK, 14-20-7 with micronutrients; Industrias Agrícolas
Unidas, Zamora, Mexico). Maize plants were maintained in a ventilated
heated greenhouse located on campus at the IIAF-UMSNH.

EFFECT ON FOOD CONSUMPTION

Newly molted (<8 h old) 3rd instars of S. frugiperda were fed with
maize leaf pieces (20 mm × 15 mm), which were obtained from expand-
ing true maize leaves that had been surface sterilized with 0.01% (wt/vol)
sodium hypochlorite followed by 2 washes with sterile distilled water.

Maize leaf pieces were dipped in 15 mL of 15 and 30% A. ochro-
leuca solutions for 5 s each. To enhance the wetting of the maize leaf
pieces, the surfactant sodium dodecyl sulfate was used at 0.01% (wt/
vol). Control maize leaf pieces were dipped in distilled water plus sur-
factant only. Treated maize leaf pieces were allowed to dry for 1 h and
then individually placed inside the cylindrical wells (each of which had a
22.1 mm bottom diameter) of 12-well Costar® tissue culture plates
(Corning®, New York, New York) containing 1 mL of 1.5% (wt/vol) agar
to prevent desiccation. The larvae were starved for 5 h to induce a
high feeding rate before they were placed individually in the wells, and
an experimental unit consisted of a group of 12 larvae in a culture
plate. In each group, 1 of the treatments was applied: 15 replicates
were treated with the 15% ethanolic extract, 18 replicates with the
30% ethanolic extract, and 18 with the control. . The maize leaf pieces
were removed after 48 h, and food consumption was measured. The
larvae were allowed to feed on the maize leaf pieces for only 48 h to
test whether their development was affected once the extract under
investigation was removed, as in Spodoptera litura F. (Lepidoptera: Noctuidae) (Arivoli & Tennyson 2013).

Food consumption was determined on each maize leaf piece on
which S. frugiperda 3rd instars had fed. Images of these maize leaf
pieces were obtained using an HP Scanjet 3370 scanner. They were
segmented using free GIMP v.2.6.6 software (GNU Image Manipula-
tion Program, http://www.gimp.org) to distinguish between intact and
consumed areas, and the resulting images were analyzed using UTH-
SCA Image Tool v.3.0.25 (Wilcox et al. 2002). The food consumption
of S. frugiperda 3rd instars was expressed as the average percentage of
maize leaf piece area consumed per larva, and it accounted for the
number of larvae that were alive at that time, which was calculated as:

\[
\text{% of leaf consumed} = \frac{\text{CAL} \times 100}{\text{TAL}}
\]

where CAL is the consumed area of the maize leaf piece and TAL is the
total area of the maize leaf piece.

EFFECT ON DEVELOPMENT

Surviving larvae from the food consumption assay were transferred
to a new plate in which each well contained approximately 8 g of un-
treated semi-synthetic diet. The larvae were checked at 24 h intervals
for pupation or until death occurred, and they were considered dead
when no movement was observed after a light touch with a brush. The
individual weights of a minimum of 20 and a maximum of 64 larvae per
extract concentration and control were determined after they were
transferred to the untreated semi-synthetic diet and 48 h later. The
larval and prepupal durations were also determined. The development
of surviving individuals was tracked, and the sublethal effects on pupae
(the formation, duration, mortality, and weight percentages) were re-
corded. To determine pupal weights, 52 male and 52 female pupae per
treatment were individually weighed 3 d after pupation.

The pupae were placed individually in the wells of tissue culture
plates as described for the consumption experiment and were exam-
ined daily for adult emergence. They were considered dead if any adult
had not emerged after 14 d. The emergence, sex ratio, and fecundity
also were assessed. The sex ratio was evaluated as the number of
males divided by the number of females + males. To study fecundity,
between 7 and 16 couples per treatment (<48 h old) were individually
placed in oviposition containers (7.5 cm in diameter × 5 cm in height)
lined with brown paper (replaced at 2 d intervals) and provided with
a 15% honey solution. The cumulative number of eggs per female was
recorded until death to compare the fecundity among treatments.

DATA ANALYSES

Data on the food consumption, larval weight, duration of the lar-
val and prepupal stages, larval and pupal mortality, pupae formation,
adult emergence, and fecundity were subjected to an analysis of vari-
ance (ANOVA) followed by least significant difference (LSD) multiple
range tests (P < 0.05) to separate the means with the Statgraphics
graphic software system (STSC Inc., Rockville, Maryland). In cases in
which the ANOVA assumptions were violated and could not be met
by transformation, a non-parametric Kruskal–Wallis test was applied.

The pupal weights were analyzed using the general linear models
(GLM) procedure, with LSD for means separation (SAS/STAT® software
version 8.1; SAS Institute, Cary, North Carolina). For the sex ratio, a
binomial distribution by sex was used.

Results

FOOD CONSUMPTION

When S. frugiperda 3rd instar larvae were fed with maize leaves
that had been treated with the 2 concentrations of A. ochroleuca
ethanolic extracts for 48 h, a slight but significant antifeedant activ-
ity (which was estimated as the percentage of leaf damage per larva)
was detected for both bioassay concentrations (19.14 ± 0.81 and 19.38
± 0.90% in 15 and 30% concentrations, respectively, compared with
22.26 ± 0.90% in the controls) (Table 1).
Immediately after the larvae were fed with treated maize leaves for 48 h, we detected a significantly lower larval weight compared with that of the control larvae when the larvae fed on 30% *A. ochroleuca* extract (3.48 ± 0.16 versus 4.53 ± 0.21 g); the weight difference remained after 48 h of feeding on an untreated artificial diet (8.56 ± 0.43 versus 13.80 ± 0.55 g) (Table 1).

### EFFECTS ON DEVELOPMENT

The 2 ethanolic extracts (15 and 30%) of *A. ochroleuca* had direct and indirect effects on *S. frugiperda* larvae. The molting process was impaired and several morphological alterations were observed; namely, we found double head capsule formation, extrusion of the hindgut, loss of hemolymph, an inability to shed the old cuticle, and cuticular blackening. In addition, shortly after treatment, some affected larvae exhibited tremors followed by paralysis and death.

Ethanolic *A. ochroleuca* extracts caused a delay in the development of larvae, prepupae, and pupae of *S. frugiperda* when the 3rd instars ingested the extract. At both concentrations (15 and 30%), we found a significantly lengthened duration of the larval stage (14.24 ± 0.81 and 19.38 ± 0.90 d, respectively) compared with controls (13.52 ± 0.06 d) (Table 1). The duration of the prepupal stage was rather similar in insects that were given treated and untreated leaves, even though a significant effect was detected at the 30% concentration (2.25 ± 0.06 d compared with 2.09 ± 0.03 d in controls) (Table 1).

The percentages of pupae that formed were not affected by the treatments, and between 93.3 ± 3.96 and 96.4 ± 1.39% of the larvae molted successfully into pupae (Table 2). By contrast, feeding on treated leaves at both concentrations significantly lengthened the duration of the pupal stage in males, which lived up to 1.37 d longer than the controls (11.50 ± 0.21 d). In females, the effect was detected only at the 30% concentration, and they lived 1.04 d longer (11.08 ± 0.22 d) (Table 2).

Ethanolic extracts of *A. ochroleuca* did not affect pupal mortality (13.51 ± 3.39 to 19.94 ± 3.88%), pupal weight (217.05 ± 3.44 to 222.27 ± 3.47 g in males and 215.63 ± 3.89 to 225.06 ± 3.88 g in females) (Table 2), adult emergence (72.12 ± 6.12 to 81.38 ± 3.8%), the sex ratio (48.8 to 51.9%), and the cumulative number of eggs laid per female during its lifetime (902.33 ± 62.49 to 1,012 ± 96.52) (Table 3).

### Discussion

Nine out of 29 living insect orders feed on the tissues of higher plants, which have developed several mechanisms to prevent damage (Panda & Khush 1995). Phytochemicals produced by secondary plant metabolism are the major measures against herbivory, and the pattern of distribution varies considerably among families (Koul 2005). Alkaloids, a heterogeneous group with one or more nitrogen atoms in a cyclic system, are present in many plants, and they have been evaluated widely because of their toxicity and phagodeterrent effects against various species (Levin 1976). The genus *Argemone* is very rich in alkaloids, which have both pharmaceutical and pest control properties. This genus is used to treat various human ailments because of its antimicrobial, antiparasitic, antimalarial, cytotoxic, and neurological properties (Rubio-Pina & Vazquez-Flota 2013). Additionally, it plays an important role in pest control. Its aqueous and organic extracts help to lessen crop damage due to the presence of sanguinarine, berberine,
Martínez et al.: Effects of Argemone ochroleuca extracts on Spodoptera frugiperda

and palmatine, among other compounds, which have antifeedant and repellent effects on a wide range of pests (Schnittler et al. 1997; Malikova et al. 2006). In this respect, high concentrations (200 to 800 mg of active ingredient per L) of ethanolic extracts from our A. ochroleuca study plant caused 50 to 60% repellence in T. castaneum (Bakhshwain & Alqurashi 2010). In our present study, 3rd instar S. frugiperda larvae that were fed with maize leaves treated with 2 concentrations of A. ochroleuca ethanolic extracts (15 and 30%) had a slight but significant antifeedant activity. This finding could explain why these larvae weighed less than controls after 48 h of consumption, at least in the case of the high concentration.

In other studies, ultra-low concentrations (0.5 to 0.80 g/cm²) of aqueous crude extracts from leaves of A. mexicana caused 80 to 96% repellency of adults of T. castaneum and the rice weevil Sitophilus oryzae (L) (Coleoptera: Curculionidae) at 1 h after application (Abdul Majeed & Abidunnisa 2011). In addition, the crude extract from leaves of this species had an antifeedant effect on the larvae of Crocidolomia binotalis Zeller (Lepidoptera: Crambidae) and S. litura (Bosch 2007).

For a given plant and insect, the effects of plant extracts are dependent on factors that are linked to the plant (the harvest season, part used, type of extract, and concentration), the insect (development stage and age), and the application method (Jenkins et al. 2003; Mallavarannan et al. 2008; Arivoli & Tennyson 2013; Kangade & Zambare 2013). The type of organic solvent that is used to extract the metabolites is very crucial; the same concentrations (≤2 mL/kg) of A. mexicana leaf extracts caused different mortality rates in Corcyra cephalonica (Stainton) (Lepidoptera: Pyralidae) 4th instar larvae when they were extracted in different solvents: the methanolic 10 to 20% extracts, the acetonic 10 to 70% extracts, the ethanolic 20 to 90% extracts, and the chloroformic 10 to 100% extracts (Kangade & Zambare 2013). Ethanolic extracts seem to act very quickly, which is a very desirable quality for halting pest damage as soon as possible.

A high concentration of ethanolic extracts from A. ochroleuca leaves (200 to 800 mg active ingredient per L) caused 43 to 78% mortality in both 3rd instars and adults of T. castaneum 6 d after treatment (Bakhshwain & Alqurashi 2010). In the present study, the ingestion of maize leaves that were treated with 30% A. ochroleuca ethanolic extracts caused 31% mortality in S. frugiperda 3rd instar larvae until just before pupation. Vidal et al. (2009) reported that lower concentration (20 to 154 mg/L) extracts from leaves of Argemone subfusiformis Ownbey (Papaveraceae) caused high mortality (90 to 100%) in the 4th instars of yellow fever mosquito, Aedes aegypti L. (Diptera: Culicidae), at 24 h post treatment. A field application of a 50% concentration of extracts from leaves of A. mexicana caused 90% mortality in adults of the stink bug Oebalus insularis (Stål) (Hemiptera: Pentatomidae) at 48 h after application (Cepero 1994). Some species, including larvae and pupae of the mosquito A. aegypti, are very sensitive to certain plant extracts, such as the ethanolic extracts from leaves of A. subfusiformis. The median lethal concentration for A. aegypti pupae at 48 h was only 9 mg/L (Vidal et al. 2009).

The morphological alterations detected in larvae in our assay during the molting process (e.g., the double head capsule, extrusion of hindgut, inability to shed the old cuticle, etc.) after the application of both ethanolic extract concentrations (15 and 30%) from A. ochroleuca are comparable to the development-disrupting action caused by ecdysone agonist compounds (Dhadialla et al. 2005; Pineda et al. 2006). The tremors and paralysis of larvae after treatment are indicative of a neurotoxic mode of action that could be attributed, at least partially, to the presence of sanguinarine because Schniteller et al. (1997) reported that this alkaloid inhibits the activity of the acetylcholinesterase enzyme due to its structural similarity to acetylcholine. Alterations in the regular pattern of movement (circular as opposed to zigzag in control

---

**Table 2. Effects (mean ± SE) of feeding Argemone ochroleuca ethanolic extracts on development parameters when 3rd instar Spodoptera frugiperda larvae were fed with maize leaves that had been treated with extracts for 48 h.**

<table>
<thead>
<tr>
<th>Extract Concentration (%)</th>
<th>Pupal weight (g)</th>
<th>Pupal mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 93.3 ± 3.96 a</td>
<td>11.50 ± 0.21 (60) a</td>
<td>19.94 ± 3.98 a</td>
</tr>
<tr>
<td>15 94.2 ± 2.46 a</td>
<td>12.57 ± 0.23 (52) b</td>
<td>11.50 ± 0.21 (60) a</td>
</tr>
<tr>
<td>30 96.4 ± 1.39 a</td>
<td>12.88 ± 0.21 (59) b</td>
<td>12.12 ± 0.22 (55) b</td>
</tr>
</tbody>
</table>

For each variable measured, means followed by the same lowercase letter are not significantly different between treatments (LSD mean separation, n > 0.05).
units) have also been described in A. aegypti 2nd instars after exposure to petroleum ether extracts of seeds from A. mexicana (Sakhthivadivel & Thilagavathy 2003).

Delays in the development of S. frugiperda larvae, prepupae, and pupae were observed in our assays when the 3rd instars ingested the A. ochroleuca extracts. Such delays were also observed by Malarvan-nan et al. (2008) in S. litura after consuming A. mexicana leaf extracts.

The occurrence of extra molts could have accounted for the results, and additional studies are under way to examine this possibility. The prolonged duration of the larval stage is a very undesirable effect, because larvae feed continuously for a longer period and more severe crop damage can be recorded. However, because the larval food consumption in both treatments was slightly less and the larvae weighed less than in the control, we can conclude that their fitness was impaired and the success of future generations could be compromised, as observed in Spodoptera exigua (Hubner) (Lepidoptera: Noctuidae) (Rodríguez-Enríquez et al. 2010) and Spodoptera littoralis Boisdoul (Lepidoptera: Noctuidae) (Pineda et al. 2006) when they were treated with methoxyfenozide, an insect growth regulator insecticide.

In our study, no long-term effects were detected because formation of pupae, pupal mortality, the sex ratio, and adult emergence of S. frugiperda were similar in the treated and control units after consumption of A. ochroleuca extracts. By contrast, under laboratory conditions when C. cephalonica larvae were fed with rice grains coated with diverse types of organic extracts from A. mexicana at concentrations of 1.5 and 2.0 mL/kg, the pupal mortality reached up to 40% (Kangade & Zambare 2013). The longer time period over which the extracts were continuously administered to C. cephalonica larvae (from 4th instar until pupation) compared with that of our study (48 h of feeding) could have accounted for this result as well as the accumulation and persistence of extracts in the larval tissue until pupal molt.

The pupal weight was not modified in our assays after feeding the larvae with extracts for a short period (48 h), but if the ingestion period is expanded, there could be effects. Malarvannan et al. (2008) reported a 20 to 37% weight reduction in S. litura pupae after the larvae during all 4 instars (4 d according to the conditions [22 ± 2 °C and 70 to 75% RH] at which the bioassays were performed by these authors) ingested extracts consisting of 10% aqueous, ether petroleum, and hexane A. mexicana leaf extracts.

There is not much information available on the effects of Argemone extracts on reproduction. A reduction of 50 to 100% in adult fecundity of S. litura has been reported after ad libitum feeding over the life span on diverse types of A. mexicana extracts (Malarvannan et al. 2008). However, after a short larval feeding period of 48 h in our study, this parameter was not affected.

In conclusion, when 3rd instar S. frugiperda larvae fed on ethanolic extracts from a mixture of dried stems, leaves, and flowers of A. ochroleuca for a short period (48 h) at the highest tested concentration (30%), we detected some lethal and sublethal effects in larvae. However, the treatment only prolonged the duration of the pupal stage and did not affect the adults (in terms of their emergence, sex ratio, and fecundity). We also found an increase in the duration of the larval stages and a lengthening of the feeding period on the crop. The magnitude of the reduction of feeding and the increase in larval mortality was low, which may be attributed to the short exposure time used in this study. More conclusive results may be observed in studies with younger larvae or longer feeding periods. However, several symptoms of molting disruption were observed, suggesting that A. ochroleuca ethanolic extracts may contain some ecdysone agonist compounds. Further studies should be conducted to isolate, assay, and identify these compounds.

### Acknowledgments

This research was supported by the Coordinación de la Investigación Científica, Universidad Michoacana de San Nicolás de Hidalgo, and it forms part of the undergraduate thesis of A.-J. A.-P. We thank Javier Madrigal (Faculty of Biology, UMSNH) for his kind help with the plant identification. The manuscript was improved with comments from Trevor Williams (Instituto de Ecología A. C., Xalapa, Veracruz, Mexico). We are grateful to American Journal Experts for reviewing the English style.

#### References Cited


Martínez et al.: Effects of Argemone ochroleuca extracts on Spodoptera frugiperda


