Organic extracts for fall armyworm (*Spodoptera frugiperda*) control in native corn (*Zea mays* L.)

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ABSTRACT
Objective: Determine the incidence of fall armyworm in corn plots and evaluate the insecticidal effect of garlic extract and rue essential oil and understand their phytochemistry.
Design/methodology/approach: The fall armyworm incidence was determined through the "five of golds" systematic sampling, composed of 20 plants per sampling point (100 plants in each plot). This assessment was conducted in 15 plots of native corn in the municipality of Cherán, Michoacán. Control bioassays were also tested, in the laboratory and field; where rue essential oil, garlic extract, cypermethrin, and an absolute control (water) were evaluated.
Results: The highest fall armyworm incidence (18%) occurred in a corn plot at Andasticua. In the laboratory, cypermethrin induced the highest fall armyworm mortality (100%), followed by rue essential oil (83%). In the field, both the insecticide and the rue essential oil reduced lepidopteran incidence. The main component of the essential *R. graveolens* oil is the semivolatyl cinnamoyl chromen (IUPAC name: 2-(3-phenylprop-2-enoyl) chromen-4-one).
Limitations of the study/implications: The present research has no major limitations.
Findings/conclusions: Both products, cypermethrin, and rue essential oil, effectively control fall armyworms in the laboratory and the field environments.

Keywords: Botanical bioinsecticides, active substance, rue essential oil.

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INTRODUCTION

The *Spodoptera* genus is present in all world agricultural regions (EPPO, 2020). Currently, the fall armyworm (*Spodoptera frugiperda*) is the pest with the greatest economic impact on corn cultivation; damaging the plant buds, the stems, when it behaves like a cutworm and can cause the loss of up to 50% of production in Mexico (Blanco *et al.*, 2014). This pest is mainly controlled with chemical insecticide methods, which cause considerable environmental damage, due to irrational utilization (Cano *et al.*, 2004). The indigenous farmers from the Cherán region, Michoacán state, Mexico, point out the need to implement more reasonable control strategies to improve the agroecological environment; on this, Alieri and Toledo (2010) discuss that the basis for achieving this is to start from experimentation processes and rescuing traditional knowledge. That is why this experiment was planned with a more environmentally friendly alternative. In this sense, two natural extracts; one from rue (*Ruta graveolens*), and the other from garlic (*Allium sativum*) were applied to counteract the effects caused by the fall armyworm. Rue extract comes from a perennial herbaceous plant, 50-90 cm high, with round stems and bluish-green foliage. It is frequently used for its medicinal properties and utilized for the control and management of the native corn crops of fall armyworm by the Indigenous community of Cherán, Michoacán state. Due to the above, the objectives were to determine the fall armyworm incidence in native corn plots evaluate the insecticidal effect of garlic extract and rue essential oil, and to know their phytochemistry.

MATERIALS AND METHODS

Study area

The Cherán community is located in the Purépecha Plateau region, 19° 38' - 19° 51' north latitude 101° 52' - 102° 08' west longitude, at an altitude ranging between 2,200 and 3,200 meters above sea level. It is limited to the northeast by Zacapu, to the southeast by Nahuatzen, to the southwest by Paracho, and to the northwest by Chilchota (Prontuario, 2009).

Fall armyworm collecting

Fall armyworms were directly collected, in corn plots, to carry out an *in vitro* bioassay at that moment, Instar L4 and L5 larvae with similar size, thickness, and colour characteristics were collected. Analytical keys were used for the insect identification (Bautista, 2006; Ruiz *et al.*, 2013).

Rue essential oil and garlic extraction

The oil extraction was conducted following the technique by Díaz (2017) with some modifications. First, the rue plants were washed with tap water and dehydrated for 25 days, until completely dry. Rue oil was obtained using the leaves and stems of the plants, by steam extraction using a Clevenger type device. The applied garlic extract was commercial (Garlic extract 87%. SA).

Fall armyworm incidence

The lepidopteran incidence was determined in 15 corn plots GPS located (Table 1). A systematic sampling was carried out, five of golds or X sampling, composed of twenty plants per point, 100 plants in total in each plot. The sampling consisted of selecting r groups of sampling units in each corner of the field and one in the center of it (Domínguez, 1992).

Fall armyworm *in vitro* bioassay

The bioassay was developed at the Phytopathology laboratory of the CIIDIR MICHOACÁN. It consisted of 3 treatments: 1. Rue essential oil (5 mL L^{-1} of water), 2.

Table 1. Location of native corn plots, sampled in Cherán, Michoacán, to determine fall armyworm incidence.

Site No.	Zone	Coordinates		Masl	Place
		Q	UTM		
1	13	0814526	2177962	2,172	Camino viejo de Paracho (El plan)
2	14	0186378	2178575	2,175	Camino viejo de Paracho (El plan)
3	14	0186052	2179000	2,173	Camino viejo de Paracho (El plan)
4	14	0186051	2180246	2,186	Camino viejo de Paracho (El plan)
5	13	0814510	2180285	2,185	Andasticua
6	14	0185638	2180167	2,180	Andasticua
7	14	0187278	2180149	2,197	Andasticua
8	14	0190957	2177243	2,366	Saricho
9	14	0192205	2177000	2,383	La Barricada
10	14	0193325	2180551	2,469	El Borrego
11	14	0185648	2178018	2,173	Camino de Paracho
12	14	0186404	2178566	2,174	Juanyan
13	14	0186019	2178999	2,172	Juanyan
14	13	0814520	2180280	2,189	Camino viejo Cheranastico
15	14	0187281	2180144	2,203	Camino Piedra parada

UTM=Universal Transverse Mercator; Masl=Meters above sea level.

20% CE Cypermethrin insecticide (1 mL L^{-1} of water), and 3. Absolute control (distilled water). Fifty-four fourth and fifth instar fall armyworm larvae were used. The design was completely randomized, with three treatments and three repetitions. Six larvae were considered per repetition, placing three per Petri dish. Subsequently, each of the treatments was sprayed on the larvae, with atomizers. Mortality was evaluated after 6, 12, 18, 24, and 30 h, and the dead larvae percentage was calculated using Abbott's formula (1925):

$$\%\ Mortality = 100\left(\%\ treated\ deaths - \%\ control\ deaths\right)/100 - \%\ control\ deaths$$

Fall armyworm field bioassay

The experiment took place in a native corn plot, in Andasticua, Cherán community, Michoacán. Four treatments were assessed: 1. Insecticide Cypermethrin 20% CE (1 mL L^{-1} of water), 2. Rue essential oil (10 mL L^{-1} of water), 3. Garlic extract (5 mL L^{-1} water) and 4. Control (distilled water). In this experiment, a randomized block experimental design was used, with 4 treatments and 4 repetitions. The experimental unit consisted of four 5 m long furrows. A surfactant dispersant was added in all treatments. Two applications were conducted in a 15-day interval.

The effectiveness degree of the treatments was determined following the Henderson and Tilton (1955) equation cited by Figueroa *et al.* (2019).

$$\%\, efficacy = 1 - \left[\frac{N_t \times M_o}{N_o \times M_t}\right] \times 100$$

Where: N_t=Larvae population in the control group before treatment; N_0=Larvae population in the control group after treatment; M_t=Larvae population in the experimental group before treatment; M_0=Larvae population in the experimental group after treatment.

Rue essential oil phytochemistry

A secondary metabolites identification of the rue essential oil was carried out via mass spectrometry in an ultra-high-resolution liquid chromatograph with electrospray ionization (UHPLC-ESI) of the Centro de Nanociencias y Micro y Nanotecnologías of the IPN.

Statistical analysis

The variables under study were subjected to an analysis of variance and the Tukey statistical test (p=0.05%), to determine the statistical significance between treatments, using the Statistical Analysis System (SAS) software.

RESULTS AND DISCUSSION

Fall armyworm incidence

Fall armyworm was observed in all sampled plots. The highest incidence, 18%, occurred in a plot located at Andasticua (site 7), the average incidence was 8% (Figure 1). This relatively low average incidence is due to some plots located in high areas, at altitudes between 2,203 and 2,469 meters above sea level, where temperatures are lower than those recorded in the lower areas. Research by Valdez-Torres *et al.* (2012) on corn cultivation determined that the average minimum threshold temperature for fall armyworms development is 8.7 °C, and their average optimal development temperature is 25 °C. No larval growth is considered possible when night temperatures are lower than 4.4 °C (Santana *et al.*, 2016) and the percentage of larval survival considerably decreases at lower than 9.8 °C temperatures (Yáñez *et al.*, 2019); which explains the previously stated.

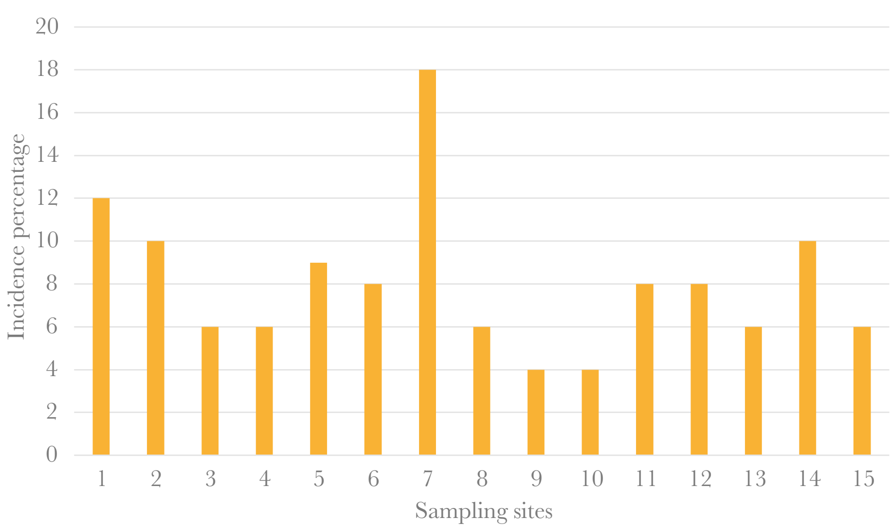

Figure 1. Fall armyworm incidence in 15 native corn plots at Cherán municipality, Michoacán state.

***In vitro* fall armyworm mortality percentage**

The chemical Cypermethrin induced the highest fall armyworm mortality, with significant statistical differences ($p \leq 0.05$). Fifty percent of the larvae treated with this insecticide died within 6 hours after its application. At 12 hours, 100% of the larvae were dead. It is reported that other chemicals, such as Spinetoram, are also capable of inducing 100% fall armyworm mortality in instars 3 and 4 (Miranda, 2016). If rue essential oil is compared with chemical treatments regarding their produced mortality, the latter may be better. However, rue essential oil can be a good prospect for fall armyworm control, because of its fewer environmental effects; contrary to insecticides that constitute a risk factor for terrestrial and marine ecosystems (García-Gutiérrez and Rodríguez-Meza, 2012). This may be because only 10% of the field-applied pesticide reaches the harmful organisms they are intended for and a proportion of them is deposited in the soil, water, and sediments (Ortiz *et al.*, 2011). In some countries, for example, Cuba, there are norms and regulations to reduce indiscriminate chemical-origin pesticide use, and on the contrary, implement biological control alternatives (Del Puerto *et al.*, 2014).

It is noteworthy that rue essential oil had anti-feeding effects for the fall armyworm, larvae treated with it stopped feeding from the first hours after spraying. Together, stopping feeding and the insecticidal effect that the essential oil could exert caused a mortality rate of 83% (Figure 2). Although the biological effectiveness of any plant extract is not 100% as in this case, these results are satisfactory. Research by Lizarazo *et al.* (2008), of fall armyworm with second instar larvae, concluded that mullein extract (Polygonum hydropiperoides) achieved 100% mortality 12 days after the larvae were sprayed. Likewise, these authors indicated that said extract had an antifeedant effect, given the less than 4% reduction in corn foliage consumption; similar results to those obtained with Chlorpyriphos insecticide. Another research demonstrated that *Solanum elaeagnifolium* aqueous extract had a mortality effect of 47.5% in a 24 to 72-hour period, in second-instar larvae exposed to that extract (Guevara, 2021). Also, the aqueous extract of *Melia azedarach* L. at a 350 g/L concentration

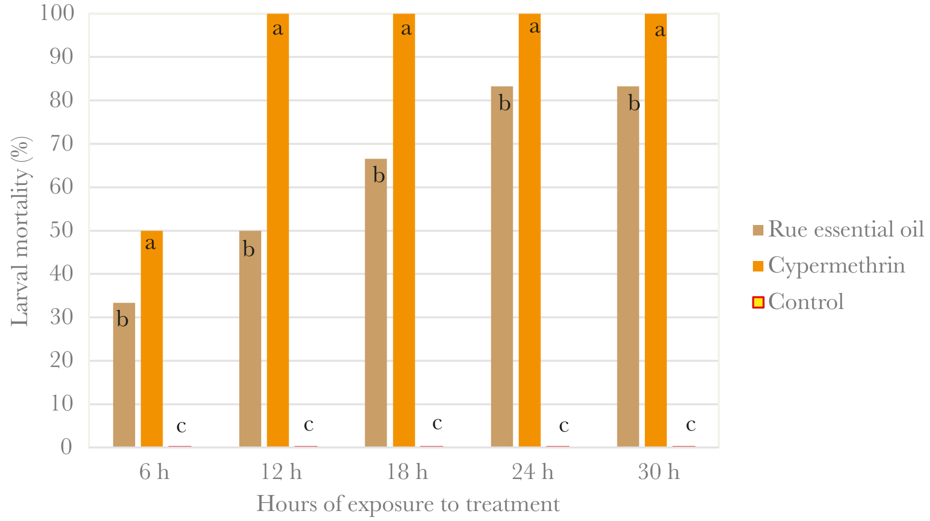

Figure 2. *Spodoptera frugiperda* larvae mortality due to treatment effects.

reported 100% mortality on the sixth day of their bioassays, as well as nutritional indices also significantly decreasing compared to their control (Mimbela, 2013).

Field fall armyworm control

The most successful fall armyworm control in the field was the Cypermethrin insecticide since it had the largest number of larvae deaths. However, it was statistically similar ($p \leq 0.05$) from the rue essential oil treatment, and this in turn with garlic extract treatment (Table 3). Other research concurs with these results and indicates that garlic extract has a biological activity against fall armyworms (Figueroa *et al.*, 2019). Likewise, these researchers point out that extracts from *Azadirachta indica*, *Piper nigrum*, *Petiveria alliacea*, *Nicotiana tabacum*, and *Lippia alba* were also effective in this pest control.

Table 2. Effectiveness degree of field treatments for fall armyworm control in native corn.

Treatments	Number of larvae (1st. Application)			Number of larvae (2nd. Application)		
	Before	After	Effectiveness (%)	Before	After	Effectivenes (%)
Cypermethrin	70	14 c	83	24	2 c	93
Rue essential oil	75	29 bc	67	41	8 bc	84
Garlic extract	71	38 b	54	50	12 b	80
Control	74	86 a	-	101	122 a	-

Different letters in the columns indicate significant differences (Tukey, $p \leq 0.05$).

It is also important to note that the fall armyworm incidence in the experimental field was not high (<15%); which may relate to the region's cooler temperature. Under optimal larval development conditions, life cycles are shorter; therefore, there may be more generations and greater dispersal in each area (Ramírez-Cabral *et al.*, 2020).

Essential oil phytochemistry

The analysis of *R. graveolens* essential oil on its secondary metabolites indicates that the main and majority component is the semivolatile cinnamoyl chromone (IUPAC name: 2-(3-phenylprop-2-enoyl)chromen-4-one) with a mass-charge ratio of 277.0831 m/z.

CONCLUSIONS

The highest fall armyworm incidence, 18%, occurred in a plot located at Andasticua. Cypermethrin, by contact, caused the 100% death of fall armyworms in 12 hours, followed by rue essential oil which reached 83% *in vitro* mortality. Both products, the insecticide, and the rue essential oil, were most effective in controlling fall armyworms in corn in the field. However, essential oils can contaminate the environment to a lesser degree, compared to the insecticide. The main *R. graveolens* essential oil component is the semivolatyl cinnamoyl chromone (IUPAC name: 2-(3-phenylprop-2-enoyl)chromen-4-one).

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