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Original article

# Insecticidal effects of *Vernonanthura nebularum* against two economically important pest insects

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ABSTRACT

*Vernonanthura nebularum* (Cabrera) H. Rob. (Asteraceae), an endemic species from the north of Argentina, is a rich source of elephantopus-type sesquiterpene lactones. These compounds have proved to be promising antiparasitic agents, but there is no report about their action against pest insects. In this work we studied for the first time the antifeedant and toxic effects of *V. nebularum* natural products against the fall army warm *Spodoptera frugiperda* Smith and the oviposition deterrent activity against the fruit fly *Ceratitis capitata* Wiedemann. As a result, we found that extracts, fractions composed of sesquiterpene lactones and pure sesquiterpene lactones altered larval feeding behavior in the food choice test. Nutritional parameters of *S. frugiperda* larvae were also affected. Fraction II (300 μg/g of diet.), containing compounds **1**, **2** and **3**, was the most toxic substance with 80% pupal mortality and wing malformations in adults. In oviposition deterrent experiments against *Ceratitis capitata*, we observed a moderate effect at 30 μg/cm$^2$ of the test compound. The most active substances were the methanolic extract, dichloromethane subextract and lactone **2**. According to our results, *V. nebularum* natural products could be used for maximizing the effectiveness and specificity in future insecticide design with specific or multiple target sites, while ensuring the economic and ecological sustainability, in addition to combat the increasing resistance rates developed by synthetic pesticides.


## 1. Introduction

It is well known that the excessive use of synthetic pesticides in the last decades has resulted in a widespread environmental pollution, toxicity to non-target organisms and negative effects on human health (Damala, 2011; Gill and Garg, 2014). Application of synthetic insecticides is not only expensive, but also counterproductive as it leads to the development of resistance by insects (Pavunraj et al., 2016).

Worldwide, about 3 billion kg of pesticides is applied each year with a purchase price of nearly U$S 40 billion (PAN-Europe, 2003). Only in the United States the cost in environmental and societal damages is estimated at U$S 9.6 billion. This assessment includes: pesticide impact on public health; livestock and livestock product losses; increased control expenses resulting from pesticide-related destruction of natural enemies and from the development of pesticide resistance in pests; crop pollination problems and honeybee losses; crop and crop product losses; bird, fish, and other wildlife losses; and governmental expenditures to reduce the environmental and social costs of the recommended application of pesticides (Pimentel and Burgess, 2014).

In the search for new strategies in crop protection, botanical insecticides and plant derived semiochemicals are being considered as good candidates for insect control and more ecofriendly alternatives (Bullangpoti et al., 2012). Plants belonging to the Meliaceae (Akhtar et al., 2008; Carpinella et al., 2003), Rutaceae (Barakat, 2011), Asteraceae (Gonzalez Coloma et al., 2005), and Annonaceae families (Ruiz Hidalgo et al., 2016) have been reported to exhibit interesting insecticidal activities. These plant families are the most promising source of terpenes, which can act as larvicides, insect growth regulators and feeding and oviposition

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deterrents (Miresmailli and Isman, 2014). Other plant secondary metabolites can also be used as lead molecules for the development of protective agents against insects, fungi and enzyme inhibitors (Céspedes et al., 2014).

Terpenes are one of the largest and most diverse classes of plant secondary metabolites. Within this group, sesquiterpene lactones (SLs) represent a group of biogenetically homogeneous natural products, and many of these molecules have been identified as the active principles of a wide variety of plants used in traditional medicine. SLs play an important role in plant protection against pathogens, herbivorous insects and mammals, and they also work as allelopathic agents (Picman, 1986). Recently, the insecticidal activities of two new SLs isolated from the fruit of *Carpesium abrotanoides* (Asterales: Asteraceae) were evaluated against a dipteran and a lepidopteran with promising results (Wu et al, 2016). Additionally, two eudesmane-type sesquiterpene lactones from *Inula helenium* (L.) (Asterales: Asteraceae) exerted growth inhibitory effects on *Spodoptera litura* (Lepidoptera: Noctuidae) (Kaur et al., 2017).

*Vernonanthura nebularum sensu stricto* (Cabrera) H. Robinson (Asterales: Asteraceae), a very rich source of SLs, is an Argentine endemic plant whose occurrence is limited to a small area in northwestern Argentina (Pollora et al, 2003). It is important to note that the SLs produced by *V. nebularum* are characteristic of some Elephantopodinae rather than *Vernonanthura* of Vernoniinae. SLs are common in the Asteraceae family, but they also occur in other angiosperm families. A few SLs have been isolated from fungi (Wang et al., 2013; Elissawy et al., 2015), liverworts (Asakawa et al., 1995; Asakawa et al., 2015) and Cupressaceae (Seaman, 1982; Sadgrove and Jones, 2014).

The Noctuidae family is the most diverse group within Lepidoptera and includes the highest number of species of agricultural importance (Caccia et al., 2014). The fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is the major pest of tropical-subtropical origin in the Western Hemisphere, being a generalist herbivore insect. In Northeastern Argentina, the FAW is the most important corn pest causing yield losses that fluctuate from 17 to 72% (Murúa et al., 2015). Control of this pest requires 2–4 applications of chemical insecticides per season (Hruska and Gould, 1997). Almost all agronomic crops in many countries are vulnerable to infestations every year by migrant populations of *S. frugiperda* moths carried by winds, causing outbreaks of this pest species. To control their populations, an integrated pest management scheme is required for efficient, low-residue and cost-effective management of FAW populations (Bullangpoti et al., 2012).

On the other hand, the influence of chemical agents on the ovipositional behavior of insects can be used to control pests as well as vector insects. There are several studies dealing with the host finding behavior of tephritids, and the influence of plant compounds on their reproductive success; the insect will oviposit or depart the fruit (Papachristos et al., 2009). The Mediterranean fruit fly *Ceratitis capitata* Wied. (Diptera: Tephritidae) causes important economic damage in the north of Argentina (Segura et al., 2006) and other countries (Szyniszewska and Tatem, 2014); therefore, new tools of insect pest management are needed and natural substances that could affect their ovipositional behavior are a good alternative (Salvatore et al., 2004).

Taking into account the great variety of plant secondary metabolites that are important in mediating interactions between plants and their biotic environment, the aim of the current study was to evaluate for the first time the insecticidal, antifeedant and oviposition deterrent effects of extracts and the major natural SLs (1–6) obtained from our collection of *Vernonanthura nebularum* (Cabrera) H. Robinson.

## 2. Experimental

### 2.1. Plant material

Aerial parts of *Vernonanthura nebularum* (Spreng.) H. Robinson were collected at the flowering stage in August 2015, near to San Francisco locality, Jujuy province, Argentina (UTM coordenates 20K 300917.26 m E 73863008.59 m S). A voucher specimen (QOIII N° 13) was deposited at the collection of Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán (Tucumán, Argentina).

### 2.2. Preparation of extracts, extraction and isolation of pure compounds

Aerial parts of *V. nebularum* (flowers and leaves, 580 g) were extracted with dichloromethane (3 × 2.5 L) at room temperature for three days, the solvent was removed at reduce pressure in a rotary evaporator to yield 18.23 g (3.14%) of crude extract (Fig. 1). The extracted material was allowed to dry and then, was successively extracted with methanol (2 × 2.5 L) to yield 14.91 g (2.57%) of crude extract.

A portion of crude dichloromethane extract (9.8 g) was suspended in ethanol (150 mL) at 60 °C, diluted with distilled water (100 mL) and extracted successively with solvents of increasing polarity: petroleum ether (3 × 70 mL), dichloromethane (3 × 70 mL) and ethyl acetate (3 × 70 mL). Ether phase, dichloromethane phase and ethyl acetate phase were concentrated separately under reduced pressure to obtain the petroleum ether subextract, dichloromethane subextract and ethyl acetate subextract respectively.

Through thin layer cromatography (TLC-Merck) tests employing *V. nebularum* extracts, pure compounds isolated previously in our laboratory as reference and Godin reagent (Wako, Japan) the highest concentration of SLs was identified on the dichloromethane subextract. This one was subjected to column chromatography over Si-gel (Merck 70–230 Mesh) using dichloromethane containing increasing amounts of ethyl acetate (0–100%) as mobile phase. Two hundred fractions were collected and pooled according to their TLC profiles. Fractions I (126–138), II (139–144), III (145–153) and IV (154–170) showing significant lactone carbonyl group absorption on the IR spectrum (FT-Perkin Elmer Series1600 equipment) at 1775 $cm^{-1}$ were processed by HPLC according to Pollora et al. (2003) procedure, using a Phenomenex C-18 column (5 μm, 10 × 250 mm) (methanol-water 8:2, 1.5 ml $min^{-1}$).

Retention times ($R_t$) of pure compounds were measured from the solvent peak. Fraction I (75 mg) yield 1 (9.6 mg, $R_t$ 11 min), 2 (5.1 mg, $R_t$ 7 min), 3 (11.2 mg, $R_t$ 14 min), 4 (7 mg, $R_t$ 24 min), 5 (5 mg, $R_t$ 32 min) and 13 mg of a complex lactones mixture. Fraction II (150 mg) yield 1 (40 mg, $R_t$ 11 min), 2 (42 mg, $R_t$ 7 min) and 3 (10.2 mg, $R_t$ 14 min). Fraction III (60 mg) yield 6 (11.4 mg, $R_t$ 5.5 min), 1 (4.2 mg, $R_t$ 11 min), 2 (16.4 mg, $R_t$ 7 min) and 3 (6.7 mg, $R_t$ 14 min). Fraction IV (50 mg) yield 1 (11.7 mg, $R_t$ 11 min), 2 (11.2 mg, $R_t$ 7 min) and three minor peaks unidentified.

For compounds identification $^1H$ NMR and $^{13}C$ NMR spectra were run on a Bruker (Germany) 300 MHz NMR spectrometer and characterized by comparison with literature data (Pollora et al., 2003).

### 2.3. Spodoptera frugiperda bioassays

#### 2.3.1. Colony and diet

*Spodoptera frugiperda* larvae were obtained from our laboratory colony started on October 2015, originally collected from corn plants (*Zea mays*) and reared on an artificial diet prepared as

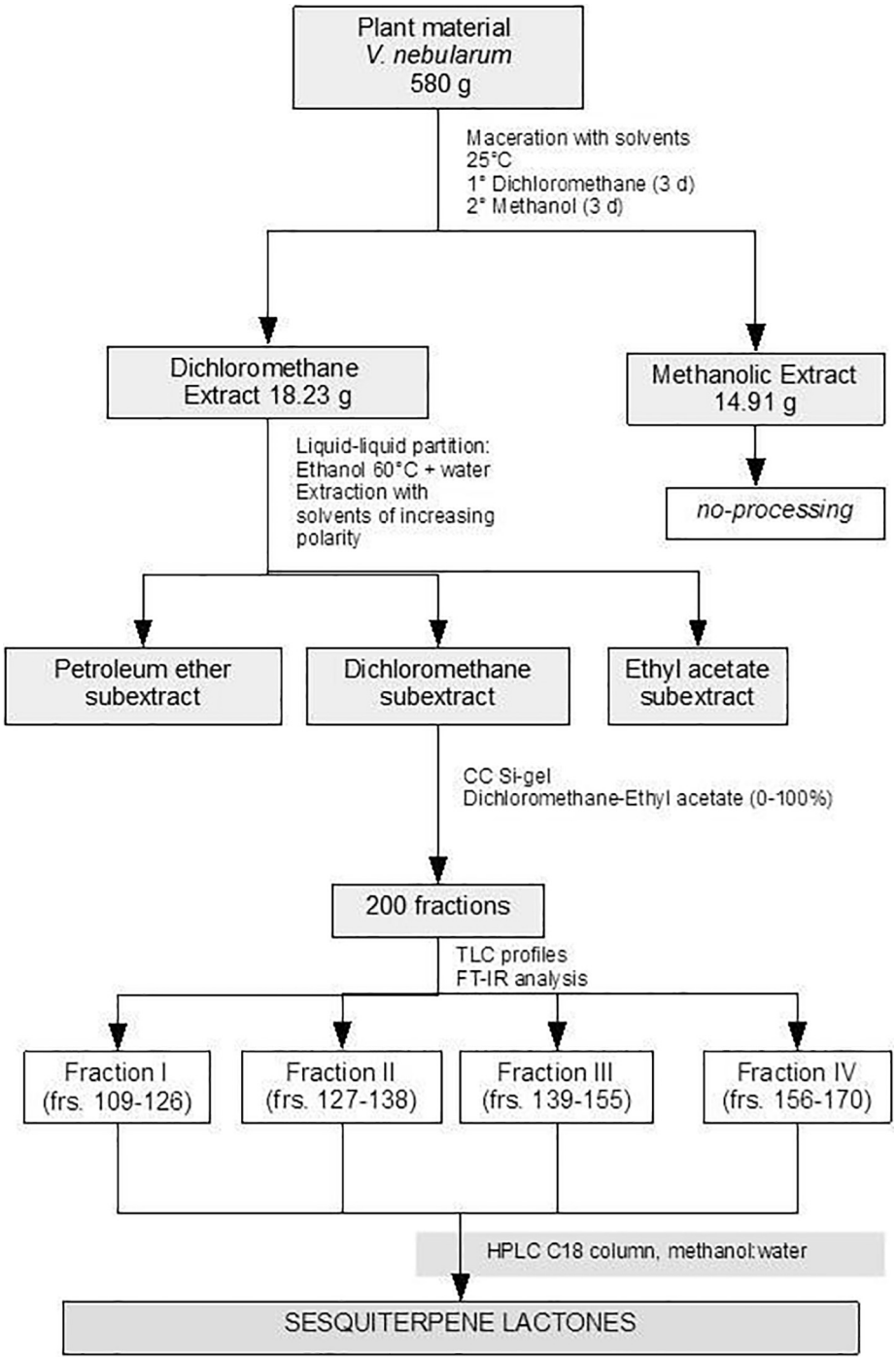



**Fig. 1.** Processing scheme of plant material.

described elsewhere (Murúa et al., 2003). Insects were maintained in a growth chamber (Ingelab I-291PF) at 24 ± 1 °C and 70–75% relative humidity, with a photoperiod of 16/8h light-dark cycle and periodically (every 3 months) renewed with field specimens (Diaz Napal et al., 2010).

*2.3.2. Choice test*

A portion of artificial diet (30 g) was mixed with an acetone solution of each test substance (treatment), in order to leave 100 μg of pure compounds and 300 μg of extract per g of diet. Another portion of artificial diet (30 g) was mixed with acetone and, after solvent removal in vacuum; this portion was employed as control diet. After solvent evaporation, 150 mg of control and treated diet were placed in a test tube. A second instar larva was placed between both portions of diet to be kept at the growth chamber under conditions mentioned above. The larva was allowed to choose the diet and, after 48 h, the remaining diets (control and treated) were weighted. The experiment was carried out in 20 replicates. To evaluate the feeding behavior an "antifeedant index" was calculated as $AI = (1 - T/C) \times 100$ (Del Corral et al., 2014), where C and T represent consumption on untreated and treated diets, respectively.

*2.3.3. Determination of nutritional indices under no choice conditions*

Second instars larvae of homogeneous size were weighed and individually placed in glass tubes. Treated and control diets

(prepared as described for choice conditions) were also weighted and offered to larvae in each tube (20 replicates for control and 20 for each treatment). Tubes were kept at 27 °C ± 1 °C in a chamber (70–75% relative humidity, with a photoperiod of 16/8h light-dark cycle). Every two days faecal matter was eliminated and every addition of diet with the corresponding weight was recorded.

At the end of experiment (ten days period), larvae were weighted and food consumption was determined. Nutritional indices, namely relative consumption rate (RCR), relative growth rate (RGR) and efficiency of conversion of ingested food index (ECI) were calculated according to Nathan and Sehoon (2006), as follows: [RGR = (A − B)/t], which gives the average of larval weight increment per hours [A = final larval weight, B = initial larval weight, and t = experimental period in hours].[RCR = D/t], is the average of the larval diet consumed per day where D is the total weight of food consumed during the experiment and t = experimen tal period in hours.[ECI = (ΔB/D) ∗ 100], where ΔB change in larval weight (mg) and D is the total weight of food consumed during the experiment.

#### 2.3.4. Toxicity bioassay

Additional observations were recorded on sublethal effects as larval, pupal and adult deformities. Lifecycle measurements, such as time to larval duration, time to pupal duration and adult emergence were measure. Finally, larval and pupal mortality were also recorded.

#### 2.3.5. Statistical analysis

Differences in the mean values were evaluated by analysis of variance (ANOVA) for one-way classification followed by a *post hoc* analysis using Dunnet's test (P < 0.05) by using Minitab®17.

### 2.4. Ceratitis capitata bioassay

*Ceratitis capitata* adults were obtained from a colony reared on Estación Experimental Obispo Colombres (Tucumán-Argentina). The colony of *C. capitata* was initiated with pupae obtained from infested oranges from the northwest of Argentina. Adults were fed on artificial diet made of water and a mixture of sugar and yeast hydrolysate (3:1). They were maintained in a rearing room with a photoperiod 12L:12D, at 24 ± 2 °C and 60 ± 10% relative humidity.

Artificial fruits (oviposition substrates) were prepared by boiling a mixture of peach juice (500 mL), agar (15 g), and sodium benzoate (2 g). This agar solution was poured into cylindrical molds, allowed to gel, and sliced (5 cm diameter × 0.5 cm thickness). The agar cylinders were then wrapped in plastic food wrap film to avoid dehydration. The surface of the wrapped cylinder was pricked with a needle and treated with an acetone solution of the sample to be tested. An amount of 30 μg/cm$^2$ of the test compound was deposited. Control cylinders were impregnated only with acetone that was then removed *in vacuo*.

Three groups of *C. capitata* adults consisting of seven male–female pairs were selected from the laboratory colony, placed on individual cage (15 cm height × 20 cm diameter) and covered with voile (a light, almost transparent cloth made of silk). Two agar cylinders (treated and control) were placed over the voile, and females oviposited on one or the other according to their preference (Socolsky et al., 2008). During the experiment, adults were fed on diet made of water and a mixture of sugar and yeast hydrolysate (3:1). After 72 h, eggs were gently rinsed from the agar and counted. To facilitate results interpretation, an oviposition index was defined as **OI** = (1 − T/C) × 100 (Socolsky et al., 2008) being T is the number of eggs laid in the treated artificial fruit, and C the number of eggs deposited in the control fruit. This index takes positive values for oviposition deterrents and negative values for oviposition attractants.

#### 2.4.1. Statistical analysis

Results are reported as mean ± SEM. Differences in the mean values were evaluated using the t test for all pair wise comparisons. In all statistical analyses, P values > 0.05 were considered not significant.

## 3. Results

### 3.1. Compounds

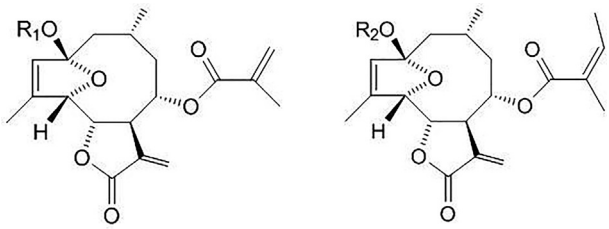
The sesquiterpene lactones isolated were six highly oxygenated elephantopus-type compounds purified by high resolution chromatographic techniques. They were unequivocally characterized through spectroscopic data previously published (Pollora et al., 2003) and new 13C-RMN data (see supplementary material). Lactones **1**–**6**, characteristic of Elephantopodiinae rather than *Vernonanthura* of Vernoniinae, carry an α-methylene- γ- lactone moiety and a furane ring in their structures (Fig. 2). Sesquiterpene lactones (**3** and **6)** were not evaluated for their activity against two pest insects because they are minority constituents.

### 3.2. Antifeedant activity

The antifeedant properties of *V. nebularum* extracts, fractions and pure compounds against *S. frugiperda* are shown in Table 1. The most active substances were the dichloromethane, ethyl acetate subextracts, fractions I and IV, with AI values greater than 75% for all of them. All the pure compounds evaluated (**1**–**2** and **4**–**5**) inhibited intake, with AI greater than 50%. It is important to note that none of the tested substances was a phagostimulant.

### 3.3. Nutritional alterations against S. frugiperda

According to the results shown in Table 2, all the tested substances influenced consumption and growth indices in treated groups compared to control. Larvae fed diets with 300 μg/g of dichloromethane subextract showed significant growth and intake inhibitions. Fraction II, obtained from the chromatographic processing of dichloromethane subextract, contains a high concentration of lactones **1** (31%) and **2** (32%) and a low proportion of lactone **4** (8%). This fraction caused the most significant reduction of RCR values, with 44% lower intake than that of the control, accompanied by a 56% reduction of RGR compared to control. Pure compounds also affected nutritional indices; however, when nutritional indices were evaluated employing fraction II, effects against larvae were more drastic compared to the test with




1. **R1=H**
2. **R1=$CH_3$**
3. **R1=$CH_2CH_3$**
4. **R2=H**
5. **R2= $CH_3$**
6. **R2=$CH_2CH_3$**

**Fig. 2.** Sesquiterpene lactones isolated from *Vernonanthura nebularum.*

**Table 1**
Antifeedant index (AI) of the assayed extracts, fractions and pure compounds from *V. nebularum* on *S. frugiperda* larvae.

| Extracts (*aerial parts*) | Doses (μg/g) | AI[a] choice |
|---|---|---|
| Dichloromethane | 300 | 52.06 ± 7.42 |
| Methanolic | 300 | 57.64 ± 11.17 |
| *Subextracts* | | |
| Dichloromethane | 300 | 76.71 ± 6.70* |
| Ethyl acetate | 300 | 86.98 ± 8.50* |
| Petroleum ether | 300 | 46.08 ± 4.21 |
| *Fractions* | | |
| I | 300 | 85.83 ± 6.65* |
| II | 300 | 60.62 ± 8.73 |
| III | 300 | 52.64 ± 8.23 |
| IV | 300 | 86.97 ± 8.51* |
| *Compounds* | | |
| 1 | 100 | 55.27 ± 8.47 |
| 2 | 100 | 67.08 ± 7.81 |
| 4 | 100 | 68.80 ± 8.01 |
| 5 | 100 | 53.61 ± 7.60 |

[a] Means (±Standard Error) within a column followed by * indicate high feeding deterrent effects.

individual compounds. The same was also observed in treatments with fractions I, III and IV.

There was a significant ECI reduction in larvae under treatments with dichloromethane subextract, fractions II and III at 300 μg/g of diet compared to larvae fed the untreated diet. The remaining treatments did not affect ECI values compared to the control group.

### 3.4. Lethal and sublethal effects against S. frugiperda

All the assayed substances showed poor larvicidal properties. A significant increase in the larval stage period was observed in the treatments with the methanolic subextract, fractions I, III and IV and compound **1**.

The highest mortality rates occurred in the pupal stage. The dichloromethane subextract produced 40% pupal mortality, while fraction II was the most active substance with 80% pupal mortality (Table 3). Pupae under this treatment presented deformities in thorax and abdomen (Fig. 3b–d) or incomplete molting larvae-pupae (Fig. 3e) and the few surviving specimens presented wing malformations in the adult stage, leading to mating impossibility. Also, it is important to point out that fraction I caused 15% larval mortality, 35% pupal mortality and 30% adult malformations**,** while fraction III showed 60% lethal or sublethal effects on the treated larvae.

When pure compounds isolated from these fractions were added to the insect diet, (100 μg/g), toxicity was significantly lower than that observed for fractions containing a mixture of the SLs. Compound **1** presented the highest pupal mortality percentage (30%). Additionally, compounds **1**, **2** and **5** produced 30%, 35% and 30% adult malformations, respectively. The deformed adults emerged with crumpled and undeveloped wings (Fig. 4b and c) compared to normal adults (Fig. 4a).

Alterations in lifecycle duration were observed in larvae reared on a diet with methanolic extract, fractions II, III, IV and compound **1**, with a small but significant delay of the larval and pupal periods.

### 3.5. Oviposition deterrent activity against C. capitata

As seen in Table 4, none of the tested substances was an oviposition attractant. The most active substances at 30 μg/cm$^2$, were the methanolic extract (IO = 64%), dichloromethane subextract (IO = 57%) and compounds **2** (62%) and **5** (55%).

## 4. Discussion

Plants have evolved a variety of defense mechanisms to reduce insect attack, both constitutive and inducible, while insects have evolved strategies to overcome these plant defenses (Rattan, 2010). The co-evolution of plants along with insects has compelled the use of natural chemical defenses for the management of insect pests. This leads to efficient built-in defense mechanisms, and thus tropical plants offer a rich and intriguing source of secondary metabolites possessing attractive pesticidal properties. These phytochemicals are mainly biodegradable and, more importantly, they are renewable (Kubo, 1993).

Plant species belonging to the Asteraceae family are known for their content of sesquiterpenes, which have been reported to serve as toxic or feeding deterrents to herbivore insects (Fraga, 2004; Prasifka et al., 2015; Kaur et al., 2017). Botanical insecticides affect insect physiology in many different ways and at various receptor sites. In lepidopteran larvae, terpenes (drimane sesquiterpenes) block the stimulatory effects of glucose and inositol

**Table 2**
Effect of extracts and constituents of *V. nebularum* incorporated into larval diet on growth, food consumption and utilization by *S. frugiperda* larvae.

| Treatment | Doses (μg/g of diet) | RCR[a] (mg mg$^{-1}$ h$^{-1}$) | RGR[a] (mg mg$^{-1}$ h$^{-1}$) | ECI[a] (%) |
|---|---|---|---|---|
| Control | | 0.84 ± 0.09 | 0.40 ± 0.04 | 45.56 ± 2.80 |
| *Extracts (aerial parts)* | | | | |
| Dichloromethane | 300 | 0.73 ± 0.08* | 0.33 ± 0.04* | 45.56 ± 0.42 |
| Methanolic | 300 | 0.67 ± 0.1* | 0.30 ± 0.05* | 44.92 ± 0.47 |
| *Subextracts* | | | | |
| Dichloromethane | 300 | 0.54 ± 0.07* | 0.21 ± 0.07* | 38.48 ± 0.83* |
| Ethyl acetate | 300 | 0.67 ± 0.04* | 0.34 ± 0.04* | 49.80 ± 3.05 |
| Petroleum ether | 300 | 0.70 ± 0.08* | 0.30 ± 0.03* | 45.63 ± 2.60 |
| *Fractions* | | | | |
| I | 300 | 0.72 ± 0.07* | 0.33 ± 0.05* | 46.70 ± 3.79 |
| II | 300 | 0.52 ± 0.01* | 0.19 ± 0.05* | 36.54 ± 0.64* |
| III | 300 | 0.62 ± 0.03* | 0.23 ± 0.06* | 37.68 ± 2.82* |
| IV | 300 | 0.71 ± 0.04* | 0.32 ± 0.03* | 44.66 ± 2.51 |
| *Compounds* | | | | |
| 1 | 100 | 0.70 ± 0.06* | 0.32 ± 0.04* | 44.79 ± 2.78 |
| 2 | 100 | 0.80 ± 0.08* | 0.36 ± 0.04* | 45.15 ± 2.47 |
| 4 | 100 | 0.73 ± 0.08* | 0.32 ± 0.08* | 43.42 ± 0.93 |
| 5 | 100 | 0.67 ± 0.02* | 0.32 ± 0.03* | 48.18 ± 3.29 |

RCR = relative consumption rate; RGR = relative growth rate; ECI = efficiency of conversion of ingested food.
[a] Means (±standard error) within a column followed by * indicate significant differences in comparison with the control group (Dunnet's test, $P < 0.05$).

**Table 3**
Effects of extract, fractions and pure compounds from *V. nebularum* on the life cycle of *S frugiperda*.

| Treatments | Larval duration[a] (d) | Pupal duration[a] (d) | Larval mortality (%) | Pupal mortality (%) | Adult malformation (%) |
|---|---|---|---|---|---|
| Control | 19 ± 0.55 | 13 ± 0.50 | 0 | 0 | 0 |
| *Extracts (aerial parts)* | | | | | |
| Dichloromethane | 19 ± 0.98 | 14 ± 1.24 | 0 | 10 | 25 |
| Methanolic | 22 ± 0.95* | 16 ± 1.32* | 0 | 30 | 10 |
| *Subextracts* | | | | | |
| Dichloromethane | 21 ± 1.20 | 12 ± 1.43 | 10 | 40 | 20 |
| Ethyl acetate | 19 ± 0.65 | 14 ± 0.65 | 0 | 0 | 30 |
| Petroleum ether | 19 ± 0.78 | 12 ± 0.76 | 15 | 25 | 0 |
| *Fractions* | | | | | |
| I | 22 ± 1.10* | 15 ± 0.79* | 15 | 35 | 30 |
| II | 21 ± 1.34 | nd | 5 | 80 | 15 |
| III | 22 ± 1.56* | 17 ± 0.89* | 5 | 30 | 25 |
| IV | 23 ± 1.56* | 16 ± 0.90* | 10 | 10 | 0 |
| *Compounds* | | | | | |
| 1 | 21 ± 0.65* | 15 ± 1.15* | 0 | 30 | 30 |
| 2 | 19 ± 0.85 | 13 ± 0.67 | 0 | 10 | 30 |
| 4 | 19 ± 0.47 | 14 ± 0.67 | 0 | 10 | 15 |
| 5 | 20 ± 1.04 | 12 ± 0.90 | 0 | 15 | 35 |

[a] Means (±Standard Error) within a column followed by * indicate significant differences in comparison with the control group (Dunnet's test, $P < 0.05$).

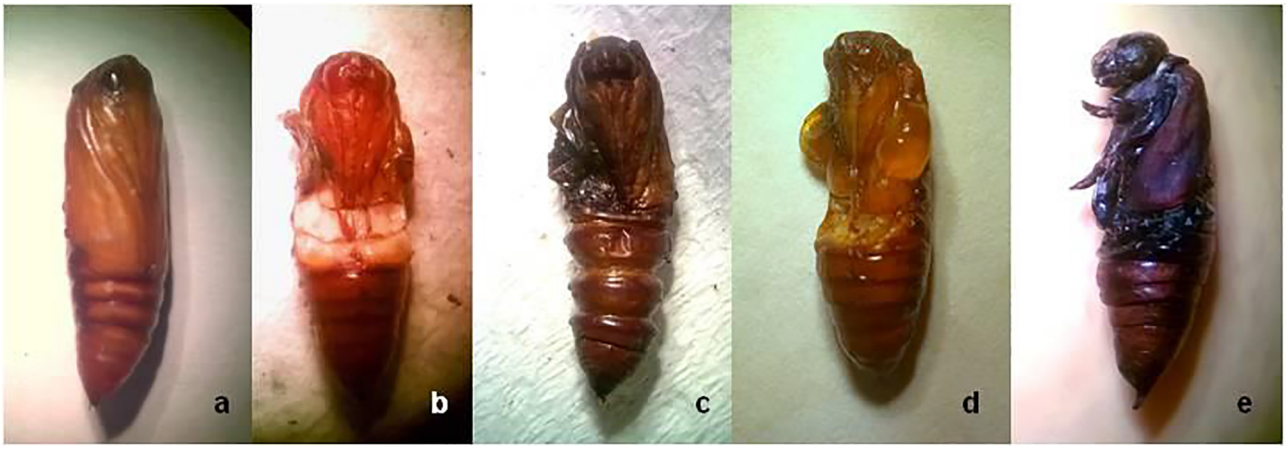



**Fig. 3.** Effects of a lactone-rich fraction of *Vernonanthura nebularum* (Fraction II) against *S. frugiperda* pupae. (a) Control pupae; (b–d) Treated pupae whit deformities in thorax and abdomen; (e) incomplete molting of larvae to pupae.

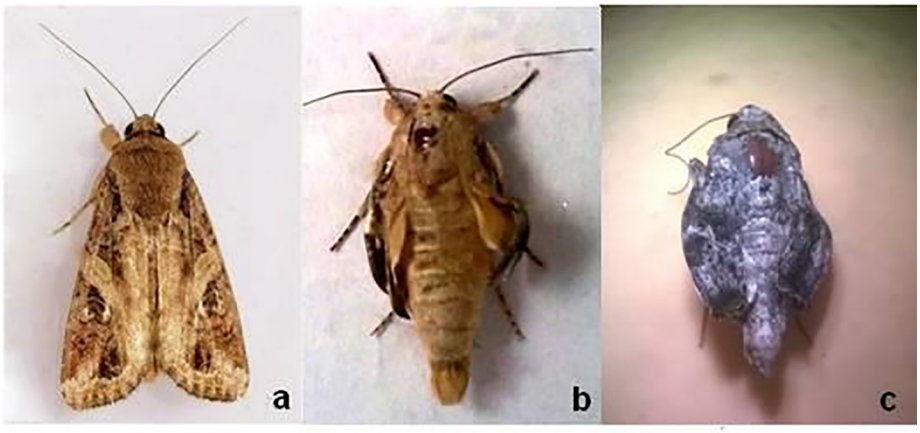



**Fig. 4.** Adults of *S. frugiperda*. (a) Normal adult (control); (b and c) Treated adult insects emerged with crumpled and undeveloped wings.

on chemosensory receptor cells in the mouth-parts, and they could also affect receptors in other ways (Gershenzon and Dudarva, 2007). Moreover, quantification of antifeedant effect of botanicals is of great importance in the field of insect pest management. From an ecological point of view, antifeedants are very important since they never kill the target insects directly and allow them to be available to their natural enemies and help in the maintenance of natural balance.

**Table 4**
Effect of *V. nebularum* extracts and constituents on the oviposition-behavior of *C. capitata.*

| Treatment 30 μg/cm$^2$ | Number of eggs laid on the control artificial fruit | Number of eggs laid on the treated artificial fruit | OI = (1 − T/C) * 100 |
|---|---|---|---|
| *Extracts (aerial parts)* | | | |
| Dichloromethane | 475 ± 25 a | 431 ± 51 a | 9.26 ± 1.40 |
| Methanolic | 687 ± 23 a | 246 ± 26b | 64.19 ± 3.82 |
| *Subextracts* | | | |
| Dichloromethane | 743 ± 74 a | 316 ± 88b | 57.47 ± 4.74 |
| Petroleum ether | 469 ± 34 a | 224 ± 59b | 52.23 ± 5.61 |
| *Fractions* | | | |
| I | 488 ± 28 a | 315 ± 39b | 35.45 ± 8.15 |
| II | 365 ± 90 a | 211 ± 56b | 51.47 ± 6.84 |
| III | 442 ± 55 a | 230 ± 56b | 42.19 ± 3.30 |
| IV | 392 ± 54 a | 289 ± 37b | 25.06 ± 7.14 |
| *Compounds* | | | |
| 1 | 357 ± 49 a | 254 ± 15b | 28.74 ± 4.86 |
| 2 | 482 ± 53 a | 185 ± 18b | 61.60 ± 5.38 |
| 4 | 474 ± 59 a | 345 ± 12b | 26.78 ± 4.86 |
| 5 | 543 ± 37 a | 240 ± 23b | 55.48 ± 10.75 |

Values represent mean ± standard deviation, n = 3. Means within a row followed by the same letter are not significantly different (P > 0.05, paired t test).

In the present work, we have explored for the first time the antifeedant, insecticidal and oviposition deterrent properties of extracts, lactone-rich fractions and pure sesquiterpene lactones from the Argentinean endemic species *V. nebularum.*

In the antifeedant assay under choice conditions, we observed that dichloromethane and ethyl acetate subextracts, fractions I and IV were the most active substances with AI values greater than 75% for all of them. Hassanali and Bentley (1987) consider inhibition is high when AI values exceed 75% and moderate when they are between 50% and 75%; therefore, all the tested substances presented moderate to high antifeedant effects under choice test conditions at the assayed concentrations. Pure SLs (**1**–**2** and **4**–**5**) have a lower antifeedant index than fractions I or IV, so it could be inferred that SLs would act synergistically. According to Rattan (2010), mixtures of terpenes containing compounds with different physical properties may be more toxic with a longer persistence of defenses. Terpenes synergize the effects of other toxins by working as solvents to facilitate their passage through membranes. On the other hand, in a previous work with lactone-rich fractions from *Cyrtocymura cincta* (Asteraceae) against lepidopteran insects (Bardon et al., 1999), the larval feeding behavior was not altered. This is probably due to the presence of a 7–11 endocyclic double bond in the lactone ring, instead of the 11–13 exocyclic double bonds present in the germacranolides isolated in this work. Additionally, it is well known that the presence of a lactone ring in the molecule is not enough to exert an antifeedant activity as it is also influenced by the presence of different oxidized groups like alcohols, ketones or epoxides (Wu et al., 2016) or by the stereochemical configuration (Paruch et al., 2000).

Under no choice conditions, intake inhibition of the tested substances was evident after ten days of treatment with second instar larvae. All the tested substances caused a significant reduction of food intake when compared to control according to the Dunnet test (P < 0.005). This behavior was associated with the significantly relative growth rate (RGR) reduction mainly observed in the dichloromethane subextract and fraction II treatments.

Under no choice conditions, inhibition of intake for the tested substances was evident after ten days of treatment with second instar larvae. All the tested substances caused a significantly reduction of food intake when compared to control according to Dunnet test (P < 0.005). This behavior was associated with significantly relative growth rate (RGR) reduction observed, mainly for dichloromethane subextract and fraction II treatments.

Therefore, according to data, constituents present in *V. nebularum* may act, like a primary antifeedant, probably via the gustatory pathway regulated by sensory organs of the mouthparts (Carpinella et al., 2003), or like a secondary antifeedant, where the reduction of food intake occurs after initial consumption and probable larval intoxication (Carpinella et al., 2003).

In treatments with the dichloromethane subextract, fractions II and III, the efficiency rate of ingested food conversion (ECI) also showed significant differences with respect to control. A decrease in the ECI values indicates that more ingested food is metabolized to obtain energy and a lower amount is converted into biomass (Rossetti et al., 2008). Deviation of energy to other metabolic pathways, such as those involved in detoxification of allelochemicals, may be the cause of the decrease in efficiencies (Koul and Isman, 1991; Hernandez and Vendramim, 1997). Such a decrease causes larval growth inhibition and is considered as a chronic post-ingestive toxic effect by several authors (Wheeler and Isman, 2001; Sadek, 2003).

In regard to sublethal and lethal effects, we observed that fraction II was the most toxic substance at the assayed concentration. Fraction II is mainly composed of lactones **1** and **2** and a small amount of lactone **4**. A high pupal mortality percentage (80%) was observed for treatment with fraction II, and the few surviving adults presented wing and abdomen malformations.

Considering the complete lifecycle of *S. frugiperda*, fractions I and III, also showed a marked toxicity affecting 60% and 55% of the treated larvae, respectively.

The pure compounds tested (**1**–**2** and **4**–**5**) showed a decrease in RCR and RGR index values with respect to control, but they did not exert significant effects on larval or pupal mortalities. Only compound **1** provoked 30% pupal mortality. SLs **1**, **4** and **5**, produced values equal to or above 30% of adult malformations at 100 μg/g of diet.

Similar results were observed when encelin, a SL containing an α-methylene-γ-lactone moiety, was incorporated into an artificial diet at a concentration of 1 μmol/g and offered to second instar larvae of *S. litura.* Larval weight gains as well as the amount of diet consumed were significantly reduced compared to controls, thus indicating an antifeedant activity (Srivastava et al., 1990). Recently, Kaur et al., (2017) evaluated the effect of alantoine and isoalantoine sesquiterpene lactones against *S. litura*, but in this case the experiment was done at a higher concentration (500 μg/g of diet). An alteration in nutritional parameters and a toxic effect on the pupal stage were observed.

The poor larvicidal properties of the assayed products may indicate that they are not acute toxins against insect larval stages, since larvae do not die quickly, although growth is affected compared to the control group.

In agreement with previous reports, although sesquiterpene lactones have been shown to act as feeding inhibitors (Mabry et al., 1977; Ganijian et al., 1983; Cis et al., 2006), we can infer that they also affect insect metabolism showing various degrees of toxicity (Kaur et al., 2017).

On the other hand, there are several studies dealing with the host finding behavior of tephritids (true flies, Diptera: Tephritidae), and the influence of plant compounds on their reproductive success. Among the most studied species is the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), one of the most notorious pests of fruit trees. Chemical and physical properties of the fruits may deter oviposition and cause egg and larva mortality conferring various degrees of resistance to respective fruit crops (Papachristos et al., 2009).

Although several works employing essential oils from plants of the Asteraceae family against *Ceratitis capitata* have been published (Clemente et al., 2008; López et al., 2011; Kurdelas et al., 2012), little is known about the use of plant extracts or sesquiterpene lactones as oviposition deterrents. According to our results, the methanolic extract of *V. nebularum* presents a promising oviposition deterrent activity against *C. capitata* (**OI** = 64%). However, the active constituents of this extract are still pending identification and would be considered for future investigations. The dichloromethane subextract, rich in sesquiterpene lactones according to the phytochemical study carried out for this report, could be considered as a good candidate for fruit fly pest control as it also presented an oviposition index close to 60%.

Among the pure sesquiterpene lactones assayed, compound **2** resulted the most active (**OI** = 62%). However, in general, these compounds were not as active in oviposition deterrence as the eudesmane-type sesquiterpenes from *Pluchea sagittalis* (Sosa et al., 2017) that our research group recently assayed against *C. capitata*.

## 5. Conclusions

Natural products from *V. nebularum* could play an important role in insect defense against *S. frugiperda*, especially when they are applied as a mixture, suggesting that a synergistic effect would be involved in the observed results. It should also be remarked that deformed adults affect insect reproduction resulting a population control. In regard to *C. capitata* bioassay, a moderate to high oviposition deterrent activity was observed. However, more studies are necessary to find the most effective concentration to improve this activity.

Although secondary metabolites from plants are sometimes commercially available as single, purified compounds, their mixture would be more effective to increase pest control and reduce pest resistance. Based on the data presented, we would like to emphasize the contribution that *V. nebularum* natural products make as tools for the development of integrated pest control strategies.

## Competing interest

The authors declare no conflict of interest.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.sjbs.2018.01.005.

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