



Feeding and oviposition of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) with sublethal concentrations of ten condiments essential oils



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ABSTRACT

The resistance, negative impacts on non-target organisms, and residues in food of synthetic pesticides necessitates the development of environmentally safe products for use in pest control. The objective of this research was to evaluate feeding and oviposition of *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) with sublethal concentrations of essential oils of condiments from ten plants, neem oil emulsion (Azamax®), and pyrethroid (Keshet® 25 EC). Bioassays were conducted in the laboratory and in a greenhouse setting using leaf disks (10.25 cm² diameter) and soybean plants in the vegetative stage V₃ with and without choice. The rate of feeding deterrence (IDF) and oviposition (IDO) of *A. gemmatalis* were calculated after 24 and 48 h of exposure to treatments, respectively. The cinnamon essential oils showed moderate antifeeding effect with IDF >50% of cinnamon mint thyme and garlic essential oils and high repellence of oviposition activity with IDO >80%. The velvetbean moth were attracted to synthetic mustard oil. The essential oils of garlic were most effective in reducing the feeding of velvetbean moth and oviposition of *A. gemmatalis* females and are therefore of interest in integrated pest management.

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1. Introduction

The use of synthetic pesticides leads to accumulation of potentially toxic substances in soil, water, and food resources (Pestana et al., 2009), which has necessitated the search for environmentally friendly and cheaper methods of pest control (Silva-Filho et al., 2014). Essential oils (EO) and plant extracts represent an alternative to pesticide for pest control (Machial et al., 2010; Fouad et al., 2014), with the advantage of high diffusion rate in the environment because of their low molecular weight and high vapor pressure (Bakkali et al., 2008).

Essential oils have lower costs, risk of environment and food contamination, and impact on beneficial organisms (Matos-Neto et al., 2002) in addition to reducing the risks of selecting

resistant insects as compared to synthetic insecticides; therefore, use of essential oils may allow a more sustainable agricultural practice (Kéita et al., 2001; Isman, 2006).

The manipulation of insect behavior with semiochemicals is one of the most promising strategies for pest control (Leite et al., 2011; Paixão et al., 2013). It can be accomplished with compounds of oviposition and feeding deterrence to reduce damage to crops by insects (Akhtar and Isman, 2004).

Essential oils from plants may have insecticidal properties such as repellency, feeding deterrence, and negative developmental and reproductive effects on insects (Ketoh et al., 2005; Isman, 2006; Souza et al., 2007), although with lesser use as insecticides, herbicides, or repellents (Isman et al., 2011). Insecticidal constituents of essential oils are mainly monoterpenes such as limonene, linalool, terpineol, thymol, carvacrol, eugenol and myrcene (Ahn et al., 1998; Chaieb et al., 2007). Thymol, the major monoterpane essential oil from thyme (*Thymus vulgaris*) is registered in Europe to control two parasitic mites of *Apis mellifera* L. (Hymenoptera: Apidae) (Rice et al., 2002). Eugenol, the main phenolic component of the essential oil from cinnamon is a broad-spectrum repellent compound

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Table 1

Plant species or trade name (Sp. Nom. Comm), common name or coach (Nom. Com Coach). Sublethal concentrations for third instar larvae obtained in preliminary tests (LC₂₅, 95% confidence interval, µL/mL)^a and botanical family (Bot. fam.) reviews of essential oils in food choice and oviposition of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae).

Sp. Nom. Comm.	Nom. Com. Coach.	LC ₂₅ ^a	Bot. fam.
<i>Allium sativum</i>	Garlic	1.23 (0.16–3.91)	Liliaceae
<i>Brassica juncea</i>	Mustard	27.10 (16.80–30.84)	Brassicaceae
<i>Cinnamomum zeylanicum</i>	Clove	9.60 (6.80–12.12)	Lauraceae
<i>Citrus sinensis</i>	Orange	58.80 (38.04–77.82)	Rutaceae
<i>Mentha piperita</i>	Mint	5.68 (3.17–8.48)	Labiatae
<i>Origanum vulgare</i>	Oregano	1.34 (0.45–2.24)	Lamiaceae
<i>Piper aduncum</i>	Black pepper	9.64 (4.45–15.10)	Piperaceae
<i>Syzygium aromaticum</i>	Cinnamon	1.30 (0.07–3.90)	Myrtaceae
<i>Thymus vulgaris</i>	Thyme	4.15 (2.21–6.18)	Lamiaceae
<i>Zingiber officinale</i>	Ginger	3.41 (0.57–7.12)	Zingiberaceae
Azamax®	Neem	0.06 (0.02–0.14)	–
Keshet 25CE®	Deltamethrin	0.005 (0.001–0.009)	–

^a Obtained in preliminary tests.

[Garden Insect Killer (64 oz)] ([Wilson and Isman, 2006](#)). Essential oils of thyme are considered safe by the Environmental Protection Agency and are therefore exempted from toxicity data ([EPA, 2004](#)).

The velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) is the main defoliating pest of soybean (*Glycine max* L. Merrill, Fabaceae) in Brazil ([Sosa-Gomes, 2004; Panizzi, 2013](#)). This species occurs in the central region of the United States to Argentina and some Indian islands ([Riffel et al., 2012](#)). *A. gemmatalis* damage other crops also, such as peanuts – *Arachis hypogaea* (L.), alfalfa – *Medicago sativa* (L.), bean – *Phaseolus vulgaris* (L.), pea – *Pisum sativum* (L.) (Fabaceae), rice – *Oryza sativa* (L.), and wheat – *Triticum aestivum* (L.) (Poaceae) ([Sturmer et al., 2013](#)). Injuries caused by *A. gemmatalis* begin with the consumption of the epidermis and mesophyll up to consumption of the entire leaf, leaving only its veins intact ([Bund and Mcpherson, 2000](#)). He upper and lower leaves are consumed the most, but the plant can be completely defoliated ([Waters and Barfield, 1989](#)).

The objective of this study was to evaluate the potential of reducing feeding and oviposition of *A. gemmatalis* with sublethal concentrations of condiments essential oils from ten plants and two commercial products in laboratory and greenhouse settings.

2. Material and methods

The surveys were conducted in the Laboratory of Biological Control of Insects (LCBI) of the Institute of Applied Biotechnology for Agriculture (BIOAGRO) and in-vegetation chamber of the Department of Plant Science (DFT) of the Universidade Federal de Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil.

2.1. Rearing pest species

Moths and adults of *A. gemmatalis* were obtained from the mass rearing facility of LCBI, where adults were placed in screened wooden cages (30 × 30 × 30 cm) covered with a paper at the top and screened from sides with glass lids (temperature of 25 ± 1 °C, 70 ± 10% RH and 12 h photophase). The adults were fed with a nutrient solution soaked in cotton at the bottom of the cages. Every 2 days, the papers with *A. gemmatalis* egg masses were removed from the cages, cut in strips (2.5 × 10 cm), and transferred to 1000-mL plastic pots containing cubes (15 × 15 × 15 cm) of artificial diet ([Greene et al., 1976](#)) for newly hatched velvetbean moth. Groups of twenty *A. gemmatalis* larvae were placed in plastic jars until pupation with proportional quantity of artificial diet for development. Food was replaced and pots were sterilized every 48 h ([Almeida et al., 2010](#)).

2.2. Obtaining botanical oils

The essential oils of garlic were acquired from Viessence Commerce Natural Products Ltda. (Porto Alegre, Rio Grande do Sul Brazil) and Ferquima Indústria e Comércio Ltda. (Vargem Grande Paulista – São Paulo, Brazil), where it is extracted on an industrial scale by hydrodistillation and dragging water vapor ([Dapkevicius et al., 1998; Santos et al., 2004](#)). Synthetic mustard essential oil used in the food industry was provided by the company Marie Fine Chemicals (Itaquaquecetuba, São Paulo, Brazil). Commercial products of Azamax® (neem) and Keshet 25CE® (synthetic chemical deltamethrin) were bought in shops specialized in agriculture.

2.3. Feeding deterrence

Discs of 10.25 cm² of soybean leaves, *Glycine max* (L.) Merrill (Fabaceae) were cut from plants in the experimental field of the UFV and immersed in 5 mL of sublethal concentration solution (LC₂₅, causing 25% mortality) of each product for *A. gemmatalis* larvae in preliminary tests ([Table 1](#)) or in ethanol (control) for 5 s and allowed to dry on paper towel outdoors for 10 min. These discs were offered to third instar (from this stage the caterpillars can start the economic damage, due to the development biting mouthparts) *A. gemmatalis* larvae (rearing on artificial diet) after 24 h fasting ([Uçkan and Sak, 2010](#)).

The phagoinhibitory properties of essential oils were evaluated by free-choice bioassays ($n=20$ per treatment) with two leaf discs set opposite in a Petri dish (12 × 1.5 cm). One disk was treated with an essential oil and the other with ethanol (one side of this disc was painted with white ink for differentiation) ([Zapata et al., 2009](#)). In the no-choice tests, one treated and one untreated disk was offered to the velvetbean moth in the arena center ($n=20$ per treatment). After 24 h, the remnants of leaf discs were collected and dried at 70 °C until constant weight was achieved in five groups, corresponding to a replicate. The food consumed was calculated by subtracting the initial from the final fresh weight of treated or untreated leaf disks ([Pavela et al., 2008](#)). The feeding deterrence index was calculated: IDF = (C – T)/(C + T) × 100; where C and T as the weights of the leaves in the control and treated ones consumed by the insect with or without choice in the laboratory, respectively ([Sadek, 2003](#)).

2.4. Oviposition repellence

Seeds of the soybean cultivar BRS Pintado, obtained from the Research Laboratory of the Department of Plant Technology from the UFV, were planted in plastic pots (10 cm mouth diameter, 15 cm × 8 cm base diameter) containing 500 mL of soil and organic compost (3:1) and with holes in their base. Plants were irrigated

daily and used in the vegetative stage V₃ (two fully developed trifoliate leaves) (Fehr and Caviness, 1977) with one and two plants per replicate in the without and with choice tests, respectively.

Pupae of the velvetbean moth from the population maintained in the Laboratory of Biological Control of Insects (LBCI) were sexed and placed in mating polyvinyl chloride (PVC) cages (20 cm × 10 cm in diameter). Two *A. gemmatalis* couples were placed after 48 h of emergence per wooden cage (30 cm × 30 cm × 30 cm) with its side covered with organza type fabric in a greenhouse with one (no-choice) or two (free-choice) soybean plants treated or not to evaluate the oviposition behavior of *A. gemmatalis*. Plants were exposed to sublethal concentrations (LC₂₅) of essential oils or commercial products containing nonionic detergent Triton X-100 (0.01%) in the mixture (Table 1) (Milano et al., 2008). The evaluation period was 48 h since copulation, and oviposition of *A. gemmatalis* under similar conditions occurred from day 1 to 2 after female emergence (Lima et al., 1998).

This experiment was conducted in a randomized block design with five replicates, with each cage containing two pairs of *A. gemmatalis* and one or two plants treated or not with essential oils or the commercial product. Each soybean plant was sprayed with 20 mL of CL₂₅ dose of the treatments (Table 1). The egg masses of *A. gemmatalis* were counted on leaves and twigs of plants treated or not with natural or commercial products 48 h after starting the experiments (Akhtar et al., 2010). The oviposition deterrence rate (IDO) was calculated with the formula: (IDO) = [(C - T)/(T + C)] × 100, where T and C are the numbers of eggs on treated or untreated plants with essential oils or commercial products (Akhtar et al., 2007).

2.5. Statistical analysis

The percent data of IDF (feeding index) and IDO (oviposition rate) were transformed into arc sine $\sqrt{x/10}$, subjected to analysis of variance, and their means were compared with Student–Newman–Keuls (SNK) ($P \leq 0.05$) by using the statistical software SAS (SAS Institute, 1997). Feeding and oviposition reduction were classified into classes of biological activity with values of IDF or IDO <0= feeding preference, IDF or IDO=0.1–30%, neutral effect, IDF or IDO=30.1–80% moderate repellence and IDF or IDO=80.1–100% strong repellency (González et al., 2011).

3. Results

3.1. Oviposition repellence

The reduction in oviposition by *A. gemmatalis* females varied with essential oils (neutral, moderate, or high), but the number of eggs of this pest was lower on plants treated with and without choice. The oviposition repellence (IDO) rates of the cinnamon, clove, ginger, mint and thyme essential oils were >80%, especially in the free-choice experiment and with similar results (Student–Newman–Keuls, $P \leq 0.05$) to those with the synthetic chemical deltamethrin (Fig. 1).

3.2. Feeding deterrence

The garlic, cinnamon, mint and thyme essential oils provided feeding repellence (IDF)>50% against third-instar *A. gemmatalis* larvae and were classified as having moderate antifeeding effect. The phagoinhibitory effect of the synthetic chemical deltamethrin was lower (Student–Newman–Keuls, $P \leq 0.05$) with and without choice, while the synthetic mustard oil showed negative IDF.

4. Discussion

A moderate to strong reduction of feeding and oviposition of third-instar larvae and females of *A. gemmatalis*, respectively, by the essential oils at sublethal concentrations can be explained by their volatile organic components that can modify the insect behavior. These effects vary with the qualitative and quantitative differences of the chemical composition of essential oils and commercial products (Akhtar et al., 2003). It may also vary with the type, harvesting time, and plant region (Hudaib et al., 2002; Formisano et al., 2013). A repellent or deterrent product contains a substance or a mixture, which is active in the vapor stage or by contact that induces the insect to move away from or avoid touching the material source (Isman et al., 2011).

The strong inhibition of *A. gemmatalis* oviposition by cinnamon, clove, ginger, mint and thyme essential oils agrees with the report of 100% value for this parameter to *Sitophilus oryzae* L. (Coleoptera: Curculionidae) with the first two essential oils, but the ginger oil did not affect the biology of this beetle (Devi and Devi, 2013). *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) did not oviposit on papers treated with *Scutellaria brevibracteata* and *Scutellaria hastifolia* (Lamiaceae) essential oils as it could detect the same with its olfactory receptors in the antennae that makes this insect avoid potentially harmful places for their offspring (Formisano et al., 2013). Antennae of *Aedes aegypti* (L.) (Diptera: Culicidae) females are sensitive to garlic essential oils (Campbell et al., 2011), and volatile oils of *Chrysanthemum morifolium* Ramat. (Asteraceae) repelled *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) females (Wang et al., 2008). These data show that cultural control with herbs such as cinnamon, cloves, ginger, mint and thyme interspaced in the plantation can reduce insect pest populations. Chemical volatiles from these plants can avoid oviposition on host plants as observed for aromatic ones such as *Ocimum basilicum* L., *Satureja hortensis* L. and *Tagetes patula* L. (Hall et al., 2008; Tang et al., 2013).

The higher reduction of *A. gemmatalis* larvae feeding by the garlic essential oils and the neutral activity of the other oils and the commercial products, except for the synthetic mustard oil, are due to compounds such as eugenol (75%) caryophyllene (8%), cinalmal (8%), γ -terpinene (3%) and other (6%), a phenolic product with diverse biological activity (Huang et al., 2002). This compound may have been primarily responsible for the moderate effect of feeding repellence (IDF>50) of essential oils as it showed neutral phagoinhibitory effect (IDF>30) and has high eugenol quantity (92.3%). Moderate phagoinhibitory effect of the cinnamon and probably other essential oils (garlic, mint and thyme) is due to components with synergistic impact between active and inactive constituents (Jiang et al., 2009). The feeding deterrence may be caused by minor compounds with low antifeeding activity of the essential oil components, as shown after the removal of borneol or β -caryophyllene terpenes from the *Lavandula latifolia* (L.) essential oil and geraniol or β -caryophyllene from the *Lavendula angustifolia* L. essential oil and the absence of methyl acetate or citronellal in the *Mentha arvensis* L. (Lamiaceae) essential oil (Akhtar et al., 2012). The caryophyllene present in the cinnamon oil and absent in that of clove has antifeeding effect (Rodilla et al., 2008), because of which *S. hastifolia* essential oil (Lamiaceae) showed this effect in *S. littoralis* (Formisano et al., 2013).

The feeding deterrence and moderate oviposition repellence of *A. gemmatalis* by the garlic essential oil components are caused by sulfide derivatives (Lawson et al., 1991). Plants of the Liliaceae family produce sulfur compounds that repel arthropods (Dugravot et al., 2004, 2005) as the diallyl trisulfide and diallyl disulfide of the garlic oil at 30–40% concentration showed longer repellence for this essential oil (Mann et al., 2011). These compounds, applied to the human epidermis, conferred greater protection against

adult *A. aegypti* than mineral paraffin oil (Campbell et al., 2011) and repelled *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) adults (Rahman and Motoyama, 2000). Other chemical components of *Allium sativum* (L.), diallyl sulfide tetrahydrofuran ($C_6H_{10}S_4$) and allicin had antifeeding effect on *Cydia pomonella* L. (Lepidoptera: Tortricidae) (Landolt et al., 1999), *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Assis et al., 2007) and *Delia radicum* L. (Diptera: Anthomyiidae) (Prowse et al., 2006).

The strong repellent and moderate feeding and oviposition deterrence of the thyme essential oil at the sublethal concentrations to *A. gemmatalis* agrees with the moderate to high repellent activity of this oil at a concentration of $10 \mu\text{g cm}^{-2}$ to *Nezara viridula* (L.) (Hemiptera: Pentatomidae) (González et al., 2011) nymphs and adults. The thyme essential oil also repelled mosquitoes (Choi et al., 2002; Yang and Ma, 2005) and other insects and mites (Tunc and Erler, 2003; Novelino et al., 2007). Furthermore, this essential oil repelled *Culicoides imicola* Kieffer (Diptera: Ceratopogonidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *T. castaneum* (Padin et al., 2000) adults.

The effect of the allyl isothiocyanate (ITCA), major component (90%) of the mustard essential oil has vapor density 3.4-times higher than that of air (Demirel et al., 2009), depending on the organism. This compound imparts phagostimulant effect on velvetbean moth and acts as gas against stored grain pests (Wu et al., 2009; Santos et al., 2011) and nematodes (Oliveira et al., 2011). Phagostimulant substances can induce the movement of insects toward a food source (Latchinsky et al., 2007), and the mustard essential oil can be used with synthetic chemical insecticides in programs of velvetbean moth control, similar to that of linoleic acid in the management of defoliator grasshoppers (Lockwood et al., 2001).

The garlic, cinnamon, cloves, ginger, mint and thyme essential oils triggered antifeeding or oviposition repellence of *A. gemmatalis* due to the fumigation activity of its compounds and thus demonstrates potential for use in greenhouses and in stored products to control insects (Jiang et al., 2012) instead of synthetic chemical products (Yi et al., 2007; Santos et al., 2010).

The antifeeding effect of garlic, cinnamon, mint and thyme essential oils with IDA (deterrence feeding effect) >50% for *A. gemmatalis* was higher than that of commercial botanical insecticide (neem) or synthetic chemical (deltamethrin). The botanical extracts of *Azadirachta indica*, *A. excelsa*, *Melia volkensii*, *M. azedarach* and *Trichilia american* (Meliaceae) were more effective to reducing the feeding of third-instar *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) larvae than pyrethrum and rotenone (Akhtar et al., 2008). The deltamethrin at sublethal concentration (LC₂₅) showed low food deterrence (IDF <30), but high oviposition repellency (IDO >80) with similar or lower oviposition or antifeeding effect than those of the garlic, cinnamon, cloves, ginger, mint and thyme essential oils. Therefore, these oils have potential in integrated pest management programs (Jiang et al., 2012).

5. Conclusions

The garlic, cinnamon, cloves, ginger, mint and thyme essential oils at sublethal concentrations can be used in integrated pest management to reduce feeding and oviposition of *A. gemmatalis*.

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