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CROP SCIENCE

Insecticidal plants as trade opportunities and use by small vegetable producers: an example using essential oils to control *Diaphania hyalinata* (Lepidoptera: Crambidae)

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Abstract: Production and sale of botanical insecticides depend on knowing the potential opportunities for these products. Essential oils from plants secondary metabolism can control pests, especially in agricultural systems where synthetic insecticides are limited, as in organic agriculture. The objective of this study was to evaluate the toxicity of essential oils to *Diaphania hyalinata* (Lepidoptera: Crambidae) and to show regions with the potential to use *Cinnamomum zeylanicum*, *Citrus sinensis*, and *Syzygium aromaticum* in the formulation and commercialization of insecticides to control this insect. The *C. zeylanicum* oil was more toxic to larvae and pupae and the *S. aromaticum* to eggs of *D. hyalinata*. Essential oils are an alternative for the management of *D. hyalinata*. The production of pesticides from essential oils of *C. zeylanicum*, *C. sinensis*, and *S. aromaticum* to control *D. hyalinata* has high potential in America. Also, Asia, Africa, Europe, the Middle East, and Asia can extract these plants to formulate insecticide molecules for the America countries.

Key words: Alternative control, *Diaphania hyalinata*, essential oil, natural products, pest control.

INTRODUCTION

Plant essential oils are mixtures of secondary compounds for different purposes, such as pest control (Regnault-Roger 1997, Tripathi et al. 2009, Pavela & Benelli 2016). Toxicity of essential oils to bacteria, fungi, insects, nematodes, mites, viruses and weeds was demonstrated, but few essential oils have become marketed products (Isman & Grieneisen 2014). This may be due to registration laws (e.g., environment, health, and trade) and economic issues such as where to produce and to whom to sell.

Essential oils may control pests, especially in crops with limitations of using conventional synthetic insecticides, such as agroecological agriculture and organic crops (Kanteh & Norman 2015). Rapid degradation of molecules in the environment or their disappearance by volatilization, are advantages usually seen in some essential oils. This minimizes contact with pollinators, biocontrol agents and non-target organisms, stimulating organic production with benefits for small farmers and agroecological sites. On the other hand, the commercialization of these possible essential oils for pest control is very limited.

Production and sale of botanical insecticides according to the worldwide distribution of species using an important Cucurbitaceae pest, *Diaphania hyalinata* (Linnaeus 1758) (Lepidoptera: Crambidae), depends on knowing the potential opportunities for these products. This pest damages the leaves, stems and fruits of plants of the Cucurbitaceae family and can cause total yield losses (Gonring et al. 2003, HansPetersen et al. 2010). The toxicity of *Cinnamomum zeylanicum, Citrus sinensis* and *Syzygium aromaticum* oils and the commercial neem product Azamax® (*Azadirachta indica*) to *D. hyalinata* eggs, larvae and pupae was evaluated.

MATERIALS AND METHODS

Compounds

The essential oils of *C. sinensis*, *C. zeylanicum* and *S. aromaticum*, extracted in an industrial scale by hydro-distillation and dragging water vapor, were purchased from the company Ferquima Indústria e Comércio Ltda, Vargem Grande Paulista, São Paulo state, Brazil. The manufacturer supplied the gas chromatography analysis for these oils (Table I). The neem oil used is the commercial product Azamax® is registered in Brazil to control agriculture pests. Immatures of *D. hyalinata* were obtained from the rearing facility of the insect biological control laboratory of the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais, Brasil.

Bioassays

The *D. hyalinata* mortality range was obtained with the bioassays to verify the oil concentrations according to the lowest (near 0%) and highest (near 100%) mortality of this insect. Essential oils + Azamax® were diluted in acetone to obtain 0.25%, 0.5%, 1%, 5%, 10% and 15% for eggs, 1%, 5%, 10%, 15% and 20% for larvae and 1%, 5%, 10%, 15%, 20% and 25% for the pupae of this insect, and the control had only this vehicle. Contact with acetone did not cause mortality of *D. hyalinata* allowing its use as a solvent.

Toxicity to eggs

The experimental design was completely randomized with six treatments (concentration), four replications, each with 20 *D. hyalinata* eggs at 24-hour-old. Fifty microliters of the essential oils (*C. zeylanicum*, *C. sinensis* and *S. aromaticum*) and the Azamax® were applied to the egg masses allowing them to air dry for 30 minutes. The eggs mass was individualized in Petri dishes (8.5 cm in diameter). The egg viability was evaluated daily for six days, sufficient time for the *D. hyalinata* hatching from viable eggs.

Toxicity to larvae

The experimental design was completely randomized with five treatments (concentration), four replications, being that each repetition consisted of 10 individualized caterpillars. Disks of pumpkin leaves (2.5 cm in diameter) were immersed, at each concentration, for 10 seconds in 10 ml of the botanical oils and that of Azamax® and air-dried for 30 minutes. One disk and one

Table I. Plant species, common name (Common), botanical family and major components (%) of the essential oils used on eggs, caterpillar and pupae of *Diaphania hyalinata* (Lepidoptera: Crambidae).

Plant species	Common	Family	Major components (%)
Citrus sinensis	Orange	Rutaceae	Limonene (95.48%), Myrcene (2.10%)
Cinnamomum zeylanicum	Clove	Lauraceae	Eugenol (75%), Caryophyllene (8%), cinamal (8%), ɣ-terpinene (3%)
Syzygium aromaticum	Cinnamon	Myrtaceae	Eugenol (92.3%), β-caryophyllene (5.50%)
Essential synthetic oil			
Azadiractina indica /Azamax	Neem	Meliaceae	Azadiractina (1.2%)

third-instar *D. hyalinata* caterpillar were placed per Petri dish (8.5 cm diameter). The mortality of these larvae was evaluated after 48 hours (Baskar & Ignacimuthu 2012).

Toxicity to pupae

The experimental design was completely randomized with six treatments, four replications, each one with 10 pupae of *D. hyalinata*. The *D. hyalinata* pupae were immersed for five seconds in five ml of the different concentrations of the essential oils and that of Azamax® and air-dried for 10 minutes. After this period, they were individualized in glass tubes (8.5 x 2.5 cm) and observed until the emergence or not of the *D. hyalinata* adults.

The toxicity bioassay was developed in a completely randomized experimental design (DIC). The treatments were represented by the concentrations of the oils and the control. The data were corrected by Abbott's formula (Abbott 1925). Concentration-mortality curves were estimated by Probit analysis and lethal concentrations (LC_{50} and LC_{90}) and their confidence limits determined.

Distribution of species

Data representing the worldwide distribution of *C. zeylanicum*, *S. aromaticum*, *C. sinensis* and *D. hyalinata* were collected from the Biodiversity Information Facility (GBIF 2019). These distributions show the potential locations for production and commercialization opportunities of essential oils to control *D. hyalinata*.

Statistical analyses

The experiment had a completely randomized design with six, five and six treatment (concentrations) for *D. hyalinata* eggs, larvae and pupa, respectively, with four replications each. Each replication had a group with 20 eggs, 10 larvae or 10 pupae of *D. hyalinata*. The regression equations and the confidence limit at 95% (Finney 1952) were calculated using the Probit analysis to obtain the LC_{50} and LC_{90} of this insect using the SAS software (Institute 2002).

RESULTS

The eggs of *D. hyalinata* were more sensitive to the essential oils of *C. zeylanicum*, *S. aromaticum* and Azamax® with LC_{50} of 1.70, 0.97 and 1.18 µL/mL and LC_{90} of 6.17, 2.38 and 15.67 µL/mL, respectively. However, with low toxicity for *C. sinensis* with LC_{50} = 40.08 and LC_{90}= 648.50 µL/mL (Table II).

The slope of the concentration-mortality for the eggs of this pest treated with *C. zeylanicum* (2.29 ± 0.52 µL/mL) and *S. aromaticum* (3.24 ± 0.48 µL/mL) oils was higher than that with Azamax® (1.14 ± 0.30 µL/mL) and *C. sinensis* (1.06 ± 0.13 µL/mL) oils (Table II).

The LC_{50} and the LC_{90} of *C. sinensis* oil and the Azamax® on *D. hyalinata* larvae were not estimated due to the low mortality of this insect in these treatments in 48 hours. The LC_{50} was 10.82 µL/mL with the oil of *C. zeylanicum* and 18.75 µL/mL with that of *S. aromaticum*. The LC_{90} for *S. aromaticum* and *C. zeylanicum* oils was 46.21 and 37.21 µL/mL, respectively (Table II). The slope of the concentration-mortality curves was 3.27 ± 0.45 µL/mL and 2.39 ± 0.44 µL/mL for the *S. aromaticum* and *C. zeylanicum* oils, respectively (Table II).

Essential oils and Azamax® have low toxicity to *D. hyalinata* pupae. The LC_{50} were 14.07, 48.92, 90.31 and 6.18 µL/mL and the LC_{90} were 269.06, 642.23, 1079.63 and 16.08 µL/ml for the *C. zeylanicum*, *S. aromaticum* and *C. sinensis* oils and the Azamax®, respectively (Table II). The slope of the concentration-mortality was 1.00 ± 0.29, 1.15 ± 0.25, 1.19 ± 0.20 and 3.08 ± 0.77 µL/mL for the oils of *C. zeylanicum*, *S. aromaticum*, *C. sinensis* and for the Azamax®, respectively (Table II).

Table II. Toxicity of the essential oils (EO) from *Cinnamomum zeylanicum* (T1), *Syzygium aromaticum* (T2), *Citrus sinensis* (T3), and the commercial product Azamax® (T4) to eggs, caterpillar and pupae of *Diaphania hyalinata* (Lepidoptera: Crambidae).

EO	LC_{50} (CI) (µL/mL)	LC_{90} (CI) (µL/mL)	X^2	Slope ± EP	P
		Eggs			
T1	1.70 (1.37-2.16)	6.17 (3.94-18.73)	6.26	2.29±0.52	0.40
T2	0.97 (0.74-1.14)	2.38 (2.00-3.11)	7.73	3.24±0.48	0.65
T3	40.08 (29.26-59.50)	648.50 (327.74-187.10)	23.48	1.06±0.13	0.17
T4	1.18 (0.53-1.69)	15.67 (7.66-126.52)	15.46	1.14±0.30	0.12
		Larvae			
T1	10.82 (6.78-14.80)	37.21 (26.56-64.99)	11.82	2.39±0.44	0.30
T2	18.75 (14.75-23.85)	46.21 (35.18-68.27)	13.66	3.27±0.45	0.19
T3	NA	NA	NA	NA	NA
T4	NA	NA	NA	NA	NA
		Pupae			
T1	14.07 (3.51-24.94)	269.06 (111.29-4849.87)	10.43	1.00±0.29	0.40
T2	48.92 (30.68-75.85)	642.23 (279.01-4718.81)	13.40	1.15±0.25	0.49
T3	90.31(63.86-126.75)	1079.63 (554.57-3771.42)	24.62	1.19±0.20	0.32
T4	6.18 (4.55-7.65)	16.08 (11.09-36.52)	4.53	3.08±0.77	0.92

NA= Not applicable (mortalities were too low to compute LC_{50} and LC_{90} value). CI= confidence interval of 95%, X^2= chi-square, SE= standard error of the mean.

The *D. hyalinata* is present mainly in the Americas, especially Central and North America (Figure 1a). The *C. zeylanicum*, *C. sinensis*, and *S. aromaticum* plants have records in all regions where *D. hyalinata* is present (Figure 1). A total of 603, 483 and 2,231 occurrences of *S. aromaticum* (Figure 1b), *C. zeylanicum* (Figure 1c) and *C. sinensis* (Figure 1d) were recorded, respectively. The presence of *C. zeylanicum*, *C. sinensis*, and *S. aromaticum* in the Americas allows the production of oil from these plants for the management of *D. hyalinata* and other pests. Also, Asia, Africa, Europe, the Middle East, and Asia can extract C. *zeylanicum*, *C. sinensis*, and *S. aromaticum* to formulate insecticide molecules for the America countries.

DISCUSSION

The high ovicidal action of *S. aromaticum* oil indicates that this is the most sensitive stage of *D. hyalinata* followed by its larvae and pupae. The wax layers in the insect egg chorion may retain toxic substances responsible for the toxicity of chemicals (Smith & Salkeld 1966, Trindade et al. 2000). However, the neem extracts and *S. aromaticum* essential oil did not affect eggs of *Tuta absoluta* (Meyrick 1917) (Lepidoptera: Gelechiidae) and *Mononychellus tanajoa* (Bondar 1938) (Acari: Tetranychidae), respectively (Trindade et al. 2000, Gonçalves et al. 2001). Differences in the impact of plant essential oils on eggs may vary between arthropod species due to their chorion structure or permeability (Gurusubramanian & Krishna 1996), reducing the impact and diffusion of toxic compounds. The penetration of products in the egg may kill the embryo, but the ovicidal effect varies with egg concentration, substance, and age (Bruce et al. 2004, Tavares et al. 2011). Larvae hatching from *Spodoptera frugiperda* (Smith 1797) (Lepidoptera: Noctuidae) eggs were lower

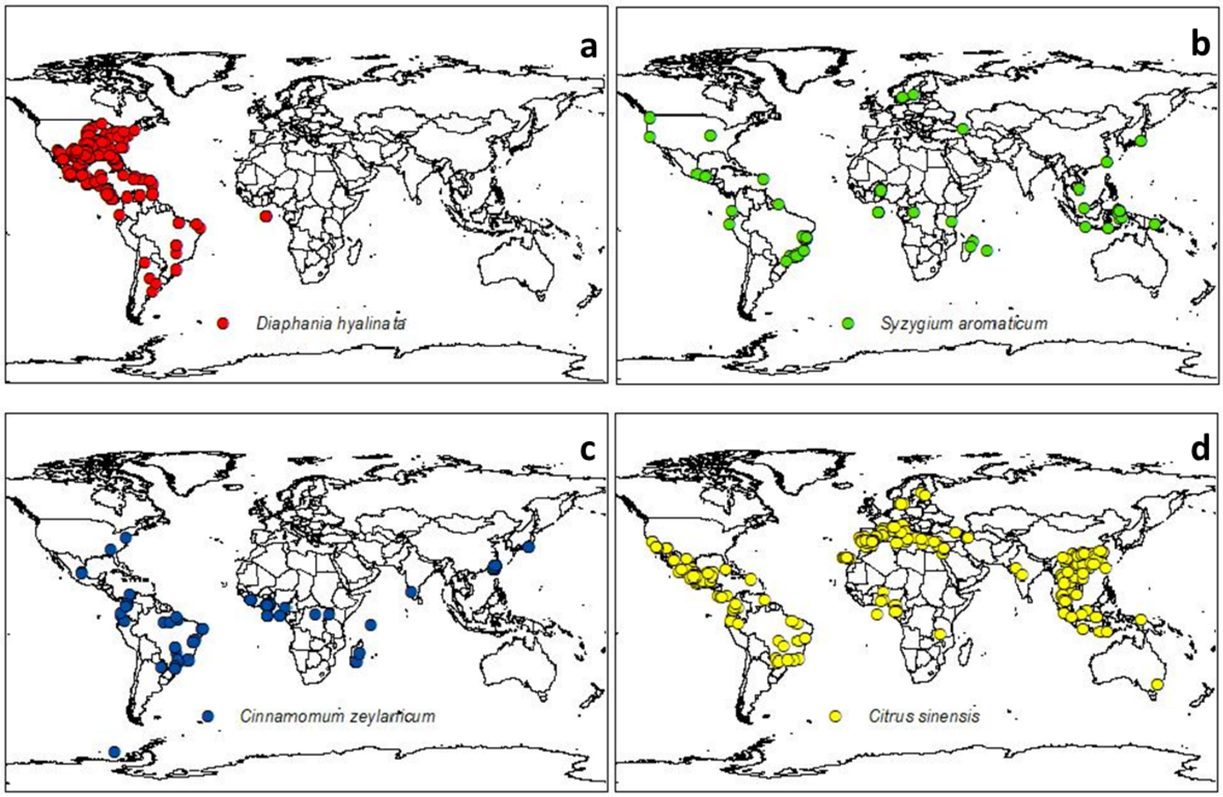

Figure 1. World distribution of *Diaphania hyalinata* (a), *Syzygium aromaticum* (b), *Cinnamomum zeylanicum* (c) and *Citrus sinensis* (d).

with 2% piperine extract than with 1% of this compound, with higher mortality of newly-laid eggs (88.80%) than those with one (36.30%) and two days old ones (15.00%) (Tavares et al. 2011).

The higher values of the concentration-mortality slope for curves of *D. hyalinata* eggs treated with *C. zeylanicum* and *S. aromaticum* oils than for those with Azamax® and *C. sinensis* indicate that low variations in the doses causes mortality variation and a higher response of eggs to increasing concentrations of oils from these plants (Kerns & Gaylor 1992).

The higher toxicity of *C. zeylanicum* and *S. aromaticum* oils to *D. hyalinata* larvae suggests that the eugenol, its major constituent, is responsible for this insecticidal action because properties of botanical insecticides are generally attributed to their major compounds (Mishra et al. 2012). Insecticidal activity of essential oils from these plants has been reported against *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae) (Birah et al., 2010) and *Trichoplusia ni* (Hubner, 1803) (Lepidoptera: Noctuidae: Plusiinae) (Akhtar et al. 2012). *Syzygium aromaticum* oil was toxic by contact and ingestion but it was lower by fumigation effect (Jiang et al. 2012). The higher slope of the concentration-mortality curve of *S. aromaticum* oil than those of *C. zeylanicum* shows higher toxicity of that product with the potential to control pests such as *S. frugiperda* (Cruz et al. 2015).

The reduced mortality of *D. hyalinata* larvae, 48 hours old, exposed to neem and *C. sinensis* oils did not allow estimating their LC_{50} and LC_{90} as the insect ceased feeding with increasing concentrations of these products. Larvae mortality with leaves impregnated with *C. sinensis* or neem oils is related to feeding deterrence caused by the secondary compounds of these products such as limonene and azadirachtin, respectively (Ruberto et al. 2002, Verza et al. 2011). Deterrents may reduce insect feeding by suppressing their appetite, as observed for *S. frugiperda* and *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) larvae exposed to citrus oils and Neemix 4.5, respectively (Ruberto et al. 2002, Charleston et al. 2006). Food deterrence may relate to primary action on insect chemoreceptors, reducing feeding, or secondary action due to physiological effects after ingestion or contact (Mordue & Blackwell 1993). Azadiractina primarily causes feeding deterrence and regulates growth, inhibiting adult molt (Mordue & Blackwell 1993), as reported for *P. xylostella* (Charleston et al. 2006), *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Eldana saccharina* Walker (Lepidoptera: Pyralidae) (Bruce et al. 2004).

The higher LC_{50} and LC_{90} for pupae than eggs and larvae of this insect suggest greater protection and that a higher dose is necessary to control the insect in these stages. This is probably due to the product intoxication pathway (contact or ingestion) and the integument structure, with greater protection of the pupae in the penetration of the products (Alvarenga et al. 2012). D-limonene kills larvae and pupae and causes the adult malformation of *S. frugiperda* (Villafañe et al. 2011). The LC_{50} and LC_{90} show that *C. zeylanicum* oil was more toxic to *D. hyalinata* pupae than those of *S. aromaticum* and *C. sinensis*. The higher slope of the Azamax® concentration-mortality curve also showed toxicity, with the greatest impact on *D. hyalinata* pupae. Essential oils inhibit or affect pupa development (Alvarenga et al. 2012), as reported for the reduced number of *Liriomyza huidobrensis* (Blanchard) adult emergence (Diptera: Agromyzidae) following exposure to *Melia azedarach* extract (Banchio et al. 2003).

Higher concentrations of *C. sinensis* oils are required to cause 50 and 90% mortality of *D. hyalinata*, regardless of its development stage. This mortality has been associated with limonene, the main component of these insecticidal essential oils (Ibrahim et al. 2001, Verza et al. 2011), which is a secondary terpenoid associated with appetite suppression or growth-inhibitory action on insects (Viegas Júnior 2003). This secondary terpenoid compound is associated with suppressing appetite or growth inhibitory action in insects (Viegas Júnior 2003).

The essential oils of *C. zeylanicum* were more efficient in the control of *D. hyalinata*, reducing the survival and the hatching of the caterpillar, with potential for use in the integrated management of this pest. The low efficiency in the control of *D. hyalinata* pupae, regardless of the essential oils used indicates that the control should be carried out in the initial stages.

The availability of plant biomass is one of the barriers to commercialization of botanical insecticides and the occurrence of *C. zeylanicum*, *C. sinensis*, and *S. aromaticum* and target pests in the same regions reduces this problem. The distribution of insecticide plant species needs further studies and this information is important to enable their use in pest management. The occurrence of *D. hyalinata* in the regions of the Americas with *C. zeylanicum*, *C. sinensis* and *S. aromaticum* as naturalized plants facilitates the extraction and the use of insecticides obtained from them. This can reduce the production and marketing costs of essential oils with insecticidal properties for the management of native pests in these regions, avoiding costs with international tariffs and the distance between demand and supply.

Essential oils are an alternative to conventional synthetic products for managing *D.*

hyalinata. The commercial Azamax® product was more toxic to the eggs and pupae of this insect. *Cinnamomum zeylanicum*, *S. aromaticum*, and *C. sinensis* oils were toxic to eggs, pupae, and larvae of *D. hyalinata*. The toxicity of these oils varies with their concentration and the development stage of *D. hyalinata*. The *C. zeylanicum* oil was the most toxic to larvae and pupae and *S. aromaticum* oil to eggs of this insect. The distribution of the studied species provides information such as opportunities for trade and the use of plant oils for managing this pest by small farmers.

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