Toxicity of two essential oils to Spotted Wing Drosophila

Toxicidad de dos aceites esenciales en la Drosophila de alas manchadas



Original Article

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ABSTRACT: There has been an increase of invasive insect species during the last decade caused by a drastic change in the biotic communities as a result of an enormous increment of the international trade and the intercontinental transportation, as well as the influence by climate change on insect species. *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) is emerging as a global threat because of its recent range expansion and the economic impact of crop fruit-colonizing populations. The aim of this work was to evaluate the toxicity effect of two essential oils obtained from plants native to Cuba on the invasive spotted wing drosophila. The susceptibility of males and females of *D. suzukii* to volatile compounds of *Thymus vulgaris* and *Piper auritum* at different concentrations was evaluated after 1, 4, and 24 hours of application. Half-maximal effective concentration (EC50) values at 24 h were calculated in each case. Both *T. vulgaris* (KD107) and *P. auritum* (KD48) oils made evident their high toxicity to *D. suzukii* males and females. The essential oil of *T. vulgaris* (107) at its highest concentration showed the highest percentage of total mortality. *T. vulgaris* essential oils could be considered as a source of bioactive substances compatible with integrated pest management (IPM) and biological control agents.

Key words: Drosophila suzukii, essential oils, susceptibility.

RESUMEN: Durante la última década ha habido un aumento de especies de insectos invasores causado por un cambio drástico en las comunidades bióticas como resultado del incremento del comercio internacional, el transporte intercontinental y la influencia del cambio climático en las especies de insectos. *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) está emergiendo como una amenaza global, teniendo en cuenta la reciente expansión en su distribución y el impacto económico en la producción de frutas debido a la colonización de sus poblaciones. El objetivo de este trabajo fue evaluar el efecto de toxicidad de dos aceites esenciales obtenidos de plantas nativas de Cuba sobre la Drosophila de alas manchadas. Se evaluó la susceptibilidad de machos y hembras de *D. suzukii* a compuestos volátiles de *Thymus vulgaris* y *Piper auritum* a diferentes concentraciones después de 1, 4 y 24 horas de aplicación. En cada caso, se calcularon los valores de concentración media máxima efectiva (CE50) a las 24 h. Tanto los aceites de *T. vulgaris* (KD107) como de *P. auritum* (KD48) hicieron evidente su alta toxicidad para los machos y hembras de *D. suzukii*. El aceite esenciale de *T. vulgaris* (107) en su concentración más alta provocó el porcentaje más alto de mortalidad total. Los aceites esenciales de *T. vulgaris* podrían considerarse una fuente de sustancias bioactivas compatibles con el manejo integrado de plagas (MIP) y los agentes de control biológico.

Palabras clave: Drosophila suzukii, aceites esenciales, susceptibilidad.

INTRODUCTION

Producing crops with high yields as a source of healthy food without damaging the environment is one of the main crisis faced by all countries in the world. A problem associated with obtaining the necessary and high production volumes to meet the demands required by food consumption is the presence of pests that can greatly reduce the productivity of the areas designed for this purpose. Based on the problems emerging from residues, potential side effects on non-target organisms, and possible resistance development in target pests, the topics being approached increasingly in pursuit of alternative solutions in crop protection are the use of insects as biological control agents of agricultural pests, natural plant products and their chemo-biodiversity, and the relationships between the production of secondary metabolites, pests and the natural enemies associated with them (1).

There was an increase of invasive insect species in the world in the past decade. In addition, in case the newly invasive species have a high adaptability and find optimal environmental conditions for a successful development in the new production systems, this may have a huge economic impact as it happened with *D*. *suzukii* in the USA and Europe (2).

*Autor por correspondencia: *Heyker L. Baños*. E-mail: hlellani@censa.edu.cu Received: 22/06/2021 Accepted: 29/07/2021 Drosophila suzukii Matsumura (Diptera: Drosophilidae) is a Drosophila species belonging to the melanogaster group. This fly is emerging as a global threat due to its recent range expansion and the economic impact of colonized populations (3) It is a multivoltine pest considered a key pest for softand thin-skin fruit crops. As suitable hosts of this fly are many commercial fruits such as blueberries, strawberries, blackberries, raspberries, some varieties of grapes, and fruits from fruit trees, such as cherries, kiwis, figs, apples, plums, peaches, among others (4). Furthermore, this invasive insect is able to feed and reproduce on a wide range of wild host plants (5).

Spotted Winged Drosophila (SWD) *D. suzukii* is native to South East Asia. It was discovered along transportation corridors in the fruit production areas in the Pacific during 2009 (6). Since then, infestations have spread across the United States, Mexico, Canada, Europe, and South America. With such a high velocity (about 1000 km per year) that kind of invasion is almost unprecedented (6,7). This rapid spread has caused severe damage to fruit production systems, resulting in millions of dollars in crop losses annually. Up to an 80 % yield loss has been reported under heavy infestations, and 20-37 % losses were estimated in incomes (2). Most recently, the fly was reported in Mexico (8,9), Brazil (10) and Argentina (11,12).

Penca, Adams, and Huler (2016), informed that, taking into account the possible opening of Cuba-USA trade and tourism, there was the concern for the introduction of new agricultural pests into Cuba, based on their location and geographical distribution. Although *D. suzukii* is not among the species reported by these authors, the risk of its entry to Cuba is real due to the growing demand of tourism and commercial trading coming from USA, Europe, and others countries of the Caribbean to Cuba.

Many essential oils have recently come into focus as repellents, antifeedants, oviposition deterrents, or toxicants for managing plant, human or animal nuisance pests. The large diversity and redundancy of phytochemicals in a single essential oil can improve control efficacy and reduce selection pressure and resistance development in pests. Natural products, including essential oils, are perceived as posing a lower risk to the environment and humans compared to synthetic compounds, although safety is dependent on biological properties of and exposure to chemicals that are not always consistent with their origin. As available management options for *D. suzukii* are currently limited, essential oils may have potential for their use in organic small fruit production systems (14).

The use of essential oils (EO) for this purpose has flourished in recent years. In Cuba, many products and extracts have been studied for their toxicity for different pests due to the potential use they may have in sustainable organic small production systems. With the objective to be prepared to reduce reliance on insecticides and improve *D. suzukii* control, it is necessary to develop new tools for its management. The aim of this work was to evaluate the toxicity and behaviour-modifying effects of essential oils obtained from Cuban plants on the invasive SWD. For this purpose, the effect of different concentrations of the essential oils of *Thymus vulgaris* (Lamiaceae) and *Piper auritum* (Piperaceae) on mortality of *D. suzukii* adults was examined.

MATERIAL AND METHODS

To evaluate *D. suzukii* susceptibility to the volatile compounds of the essential oils from *Thymus vulgaris* (KD107) and *Piper auritum* (KD48), ten adults (five females and five males) with approximately one week of age were tested. The essential oils were obtained by the Chemical Ecology laboratories of the Division of Plant Health of the National Center for Animal and Plant Health, Cuba. The insect adults were taken from a permanent laboratory colony at the JKI in Dossenheim, Germany maintained according to Eben *et al.*, (2020). They were picked up from the colony with a manual vacuum exhauster, placed in 45 ml plastic tubes with screw caps and kept on ice for 5 min. The experiments were carried out at the JKI in Dossenheim, Germany.

Each essential oil was diluted with reagent grade acetone to concentrations of 0.125, 0.25, 0.5, and 1% and a final volume of 12 ml. Acetone was used as the control.

A dismountable test arena, built according to Jacas and Viñuela (16), was used. It consisted of a transparent plexiglass ring (10 cm diameter, 3 cm height, with 4 operating holes) enclosed by glass plates (12 cm 12 cm 5 mm) on top and bottom. In one of the operating hole, it was placed a 2.5 ml Eppendorf tube without the tip and containing cotton soaked with a solution of water and sugar (5 %). The other holes were plugged with a stopper, one of the glass plates (lid of the test arena) was sprayed with 200 µl of the essential oil with the aid of a sprayer bottle (Figure 1), and allowed to dry at room temperature. The adults of D. suzukii were released in the open arena and the cage quickly closed taking care that the applied surface was placed into the area enclosed by a plastic ring. Each oil concentration was replicated five times. The set of pieces was fastened with a clothespin. The cages were placed in a laminar air flow cabin at an average of 24°C, 76 % relative humidity, and natural photoperiod (16 h: 8 h).

Mortality was recorded at 1, 4, and 24 hours after the application of the oils. The most effective essential oil and its concentration was determined by ANOVA analysis and Tukey test ($p \le 0.01$) after transforming mortality data into Arcsin \sqrt{x} (17). Half-maximal effective concentration (EC50) values at 24 h were calculated by probit regression analysis of values using SPSS v22 (IBM, USA).

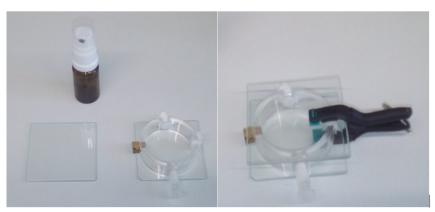


Figure 1. Materials used during the experiment (a) and experimental design (b)./Diseño experimental y materiales usados durante el experimento.

RESULTS AND DISSCUSION

The volatile components of both essential oils showed higher toxicity effects on SWD adults than the control. SWD males showed evidences of high susceptibility to both oils from *Thymus vulgaris* (KD107) and *Piper auritum* (KD48). The concentration of 1 μ l/ml was the most effective in each essential oil at 24 hours, although *Thymus vulgaris* (KD107) oil at 0.5 μ /ml showed mortalities higher than 50 % from the fourth hour of evaluation (Table 1). This is indicative of a quick insecticidal effect. In the case of P. *auritum* oil, the insecticidal effect at 24 h can be explained by the cumulative toxicity effects of some of the components.

The chemical composition of *P. auritum* essential oil, mainly that obtained from the plant aerial part, has been studied by steam distillation in several countries (18). This author proposed *Piper*-amides as the most representative compounds of the Piper species. These amides are recognized not only by their toxicity and their synergistic effects on the insecticidal activity,

but also by the important antifungal and antibacterial character they have (19).

Safrole is the major constituent of the essential oil of several species in the family Piperaceae, and its proportion can vary between 70-94 % according to the species. In Cuba, safrole was found to be the major component (74-29 %) of the essential oil of P. auritum (KD48), with different proportions of γ -terpinene (6,21 %), α -terpinolene (4,96 %), β -pinene (2,99 %), α -terpinene (2,65 %), α -pinene (1,79 %), and β -caryophyllene (1,43 %) (20). Safrole can act synergistically with other compounds present in the plant increasing their biological activity (18,19).

It is possible to associate the strong lethal activity of this plant oil with its high content of safrole, but the possibility of the whole composition of this oil at its higher concentrations in determining the high effectivity on *D. suzukii* cannot be underestimated. There is not enough information available regarding the lethal effect of essential oils from *P. auritum* plants on insects, but some ethanolic extracts of *P. auritum* were evaluated on immature stages of the potato/toma-

 Table 1. Insecticidal effects of volatile components of essential oils on Drosophila

 suzukii./Efecto insecticida en Drosophila suzukii de los compuestos volátiles de aceites esenciales.

Essentials oils	Ν	Concentration (µl/ml)	Mortality (%) ± SE		
			1 Hour	4 hours	24 hours
Piper auritum (KD48)	50	Control	0±0,00aA	0±0,00aA	0±0,08aA
	50	0.125	2±0,28abA	4±0,42aA	8±0,39aA
	50	0.25	6±0,26abA	4±0,06aA	26±0,19abA
	50	0.5	0±0,40aA	4±0,46aAB	46±0,37abB
	50	1	6±0,14abcA	8±0,40bB	100±0,08cB
Thymus vulgaris (KD107)	50	Control	0±0,00aA	0±0,00aA	4±0,00aA
	50	0.125	46±0,06abcA	48±0,06aA	48±0,06aA
	50	0.25	14±0,13bcdA	22±0,13aA	28±0,33abA
	50	0.5	54±0,00cdA	55±0,21abA	74±0,37bcA
	50	1	90±0,10dA	96±0,00bA	100±0,00cA

*Means in the same column with no lowercase letters in common differ significantly ($p \le 0.01$) (difference between treatments). *Means in the same row with not no capital letters in common differ significantly ($p \le 0.01$) (difference between times). /*Medias en la misma columna con letras minúsculas difieren significativamente ($p \le 0.01$) (differencias entre tratamientos). to psyllid *Bactericera cockerelli* (Sulc) with promising results. Alcoholic fractions of this oil caused high mortality (66 %) of adults of the whitefly *Trialeurodes vaporariorum* Westwood (21).

In the case of the essential oil of *Thymus vulgaris* (KD107), the main components are thymol (56,98 %), its biosynthetic precursor p-cimene (14,67 %,) and γ -terpinene (14,13 %). Other components found with more than 1 % were α -terpinene (1,65%), L-linalool (1,64 %), α -tujene (1,52 %), myrene (1,38 %) and carvacrol (1,33 %) (22).

The contact or fumigant toxicity of the essential oil from *T. vulgaris* and others plants within the same genus has been studied with positive effect on several species of insects, including the whitefly *Bemisia tabaci* Genn. (23), the lepidopteran *Ephestia kuehniella* and *Plodia interpunctella* (24), and the mealybug *Phenococcus solenopsis* (Tinsley) (25). It has also been demonstrated that the oils of this plant can cause complete reduction or inhibition of feeding insects belonging to orders such as Lepidoptera, Coleoptera, Hemiptera, and Orthoptera (26).

In accordance with Isman and Grieneisen (2014), the discrepancies related to the composition of the essential oils, the content of components present as major or minor constituents, and even the effects on insects can be explained by considering variations in the ecological conditions (climate, soil type, season, geographical location) in which the plant has developed. Furthermore, the extraction conditions (extraction method, time, conditions of the raw material) can produce oils that may differ in both qualitative and quantitative content.

The most effective concentrations of volatile compounds of the essential oil KD48 were in the range between 0.79-0,86 %, causing 99 % of the SWD population mortality (Table 2). However, respecting the essential oil KD107, it was necessary a concentration of 0,79 % of volatile compounds to cause the death of 99 % of males in a population.

In the last five years, there was an increase of the public interest in the use of essential oils for controlling *D. suzukii* populations. This can be explained by the increasing demand of organically grown fruits since, as it is well known, the insecticides based on natural products are perceived by the public as a lower risk to the environment and humans compared with synthetic compounds (28).

In this sense, Renkema *et al.* (14) informed that Thyme oil from the chemotype of *Thymus vulgaris* was unique among the 12 oils tested because of the higher mortality of male flies it produced compared with the other oils, and it reduced the number of responding males and females. Similar studies on strawberries showed thymol as the most effective essential oil that reduced fly landing and larval infestation and increased the SWD mortality under laboratory conditions. Also in the field, this compound can reduce the larval infestation levels by 25 % at four days after application (28).

Park et al. (29) evaluated the toxic and fumigant effect of twelve essential oils obtained from Lamiaceae plants on D. suzukii. These authors found that essential oils of Thymus zygis and Satureja montana and their components, thymol and carvacrol, exhibited contact toxicity activity and could be applied directly to control SWD; inhibition of acetylcholinesterase (AChE). was also observed. In another study, Park et al. (2017) tested the toxicity effect of six essential oils from Myrtaceae plants. In this case, the essential oils from kanuka (Kunzea ericoides J. R. et G. Forst) and manuka (Leptospermum scoparium (A. Rich.)) and their β -triketone components exhibited high contact toxicity to the fly. According to this author, pesticides based on essential oils from kanuka and manuka plants could be used to protect postharvest fruits, and the oils based on T. zvgis and S. montata could be applied directly to control SWD.

The presence of thymol as a major component may be directly linked to the insecticidal effect of the oil due to the numerous previous reports on the insecticidal action of this monoterpenic alcohol (31). This substance has many biological targets and its action mode can vary even in different insect orders and families (Carayon et al., 2014); for example in Drosophila sp., thymol can strengthen the response of RDL-type GABA receptor associated with the insect olfactory learning (34); it can inhibit the TRPL channel (some of these channels are associated with sensations like pain, hotness, warmth or coldness, different kinds of tastes, pressure, and vision) (32); and it also showed an inhibitory action on AChE (29). Bonnafé et al., (35) informed that the specificity of the response to the conditioned stimulus (CS) was lost in bees previously exposed to thymol (10 or 100 ng/bee) 24 h after learning.

Essential oil	Fly sex	Ν	Slope ±SE	Ec 99 (%)*	99%CL	X ² a/P
KD48	Female	50	4,84±0,84	0,86	0,71-1,17	2,67/0,45
	Male	50	$5,22{\pm}0,88$	0,79	0,51-2,1	5,44/0,14
KD107	Female	50	4,14±0,66	1,12	0,78-3,34	6,70/0,08
	Male	50	5,94±1,06	0,79	0,59-1,05	4,6/0,2

Table 2. Half-maximal effective concentration to Drosophila suzukii /Máxima concentración efectiva para Drosophila suzukii.

*Ec 99 is the effective concentration required to kill 99 % of the population.

In this study, it could not be differentiated whether the toxicity of the essential oils was due to the contact or to the fumigant effect. Unfortunately, even when evaporation of the complete substance was tried, not all the components had the same volatility, and thus, some part of them could still come in contact with the insect antenna, body or legs.

It is important to emphasize that despite the effect shown by both oils under laboratory conditions, their behaviour may be different in the field because of the possible loss of some of their components due to their high volatility. However, under field conditions these losses can be corrected by nano-encapsulation and/or the application of nanoparticles loaded with these essential oils. Studies in this regard have advanced rapidly in recent years (36). The effectiveness of these compounds, alone or in combination with other substances, has been tested both in the laboratory and under field conditions and against different crop pests (37-41) According to Khoobde et al. (37), when the nano-encapsulated essential oil technique is used, it can produce pesticides that have controlled-release properties and reduce the concentration of the applied doses and number of applications. Additionally, nanoformulation of essential oil saved its insecticidal property for longer time and improved its efficiency in pest control (39) and product stability (41). Furthermore, nanoencapsulated essential oils have a lasting residual effect as compared to the free agrochemicals (42).

Numerous authors evaluated the insecticidal activity of essential oils and their components on different insect species. In some cases, they had a dual activity, both fumigant and by contact. These type of dual activity is reported for different species of insects, among them the whiteflies *Trialeurodes vaporariorum* (43) and *B. tabaci* (44).

To possess high levels of activity against the selected biological target, combining several effects and toxicity to more than one of its states, is one of the most important criteria for a compound to be selected in the initial stage of research and continue its development until obtaining a possible product. Also, the toxicity of the insecticides can differ for the developmental stages of an insect, and the control of the immature states is considered as one of the most effective means for the reduction of populations of agricultural pests (45).

Drosophila suzukii Matsumura (Diptera: Drosophilidae) is still not found in Cuba, but its presence in some countries in the near-by area is known. Taking into account the influence of trade and climate change on pest movement, being prepared for the struggle against the presence of new pests in a country is crucial to reduce the impact of their introduction in agricultural areas, to reduce crops losses and their impact on fruit production. Thus, T. vulgaris essential oils could be considered a source of bioactive substances compatible with integrated pest management (IPM) biological control agents. However, a lot of research is still needed to be done. It is necessary to study the interactions between candidate essential oils and natural enemies, as well as to find the best approach for implementing this essential oil in a pest management strategy (46).

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