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Toxicity of some essential oil formulations against the Mexican fruit fly *Anastrepha ludens* (Loew) (Diptera: Tephritidae)

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ABSTRACT

Essential oils (EOs) extracted from *Eugenia caryophyllus*, *Ocimum basilicum* and *Thymus vulgaris* were evaluated for toxicity against adults of the fruit pest the Mexican fruit fly, *Anastrepha ludens* (Diptera: Tephritidae). The chemical composition of the EOs was also determined. The EOs of *E. caryophyllus* and *O. basilicum* contained primarily phenylpropanoids (77.58% and 72.63%, respectively), which were followed by benzoate esters (10.99%) and sesquiterpenes (6.22%) in *E. caryophyllus* and monoterpenes (16.65%) in *O. basilicum*. The EO of *T. vulgaris* was composed primarily of monoterpene hydrocarbons (89.39%). In ingestion toxicity assays, the EO of *E. caryophyllus* was the most toxic, with an LC50 of 3529 ppm, followed by the EOs of *T. vulgaris* and *O. basilicum* with LC50s of 5347 and 8050 ppm, respectively. At the highest concentration (1.5, 2.0 or 3.5% w/v), 100% mortality was observed with the three EOs in the ingestion toxicity assays. In general, the three EOs were significantly toxic to adult *A. ludens*. The development of a technology to incorporate the EOs into food bait could provide an alternative method to attract and kill *A. ludens* in field applications.

1. Introduction

In Mexico, as in other countries, fruit flies are a significant problem that can cause losses of fruit of 37%, increase marketing and production costs, affect the quality of the product and increase environmental pollution with the use of insecticides such as malathion for control of the pest. Fruit flies are also the cause of quarantine restrictions that limit access to production by international markets (Aluja and Mangan, 2008). The Mexican fruit fly, *Anastrepha ludens* (Loew), occurs in most citrus-growing areas in Mexico and damages, among others, two of the most important fruits, i.e., orange and mango (Aluja and Mangan, 2008). Unlike the other citrus regions of Mexico, the problems caused by the Mexican fruit fly in the northeastern region are more severe because the fly and one of the most important wild hosts, the yellow chapote *Casimiroa* (=*Sargentia*) *greggii* S. Wats (Rutaceae), are native to this region. The primary damage in citrus that causes crop losses by the Mexican fruit fly is fruit drop as a result of the internal growth of larvae. To avoid this damage, a number of phytosanitary measures must be applied, which when combined, increase crop production costs. Therefore, new alternatives are required to improve or revamp the current strategies used for the control of Mexican fruit fly populations, in addition to designing programs to prevent the dispersal of the fruit fly based on control with integrated pest management. Plant extracts are potential alternatives to currently used insecticides, and encouraging results have been obtained in several studies with some plants that have toxicological effects on flies in the family Tephritidae (Canale et al., 2013; Benelli et al., 2012). Plant essential oils are well known to have pharmacological properties, including bactericidal, fungicidal, and antioxidant activities (Kalemba and Kunicka, 2003), and the secondary plant metabolites of these oils can also act as defense mechanisms against insect herbivores.

To further our understanding of the biological effects of the essential oils of *Eugenia caryophyllus* (Spreng), *Ocimum basilicum* L. and *Thymus vulgaris* L., the following objectives were defined in this study: (1) determine the insecticidal properties of the oils against two-day-old *A. ludens* adults; (2) determine the LC50s and LC90s of the three essential oils; and (3) determine the chemical composition of each essential oil.

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2. Materials and methods

2.1. Essential oils

The essential oils (EOs) of *E. caryophyllus*, *O. basilicum* and *T. vulgaris* used in this study were commercial samples from Oils4life Limited (Norfolk, UK). The clove bud essential oil originates in Indonesia and is extracted by water distillation of the buds and leaves and by steam distillation of the stalks or stems. The basil essential oil originates in India and is extracted by steam distillation of the flowering herb. The thyme essential oil originates in Iran and is extracted by steam distillation of the fresh leaves and flowering tops.

2.2. Gas chromatography

Qualitative analysis was conducted using a Hewlett–Packard Gas Chromatograph Model 5890 series II (Hewlett–Packard, Palo Alto, CA, USA), coupled with a Hewlett–Packard Model 5972 Mass Selective Detector. Separation was conducted in a polyethylene glycol FFAP (50 m × 0.2 mm ID × 0.33 μm film thickness) capillary column. Injector and mass spectrometer (MS) interfaces were processed at 250 °C and 280 °C, respectively. The initial oven temperature was 75 °C, and the temperature was then increased to 220 °C at a rate of 2.5 °C/min, which was maintained for 10 min. The carrier gas was helium with a flow rate of 0.8 ml/min, and the injection volume was 0.1 ml using a 1:200 split ratio. The mass spectrometer was operated in SCAN mode with a 70 eV ionization voltage, with scanning from 30 to 350 *m*/*z* at 0.81 scan/s. Volatile compounds were identified using the following criteria: (1) comparison of mass spectra with the Wiley library spectra 275 L (Rev. C.00.00) electronic database; (2) injection of authentic compounds from Sigma–Aldrich with a 95% minimum purity under the identical analytical conditions; and (3) comparison of the retention index in similar phases, as reported in the literature. The volatile compounds in the essential oils were quantified by gas chromatography (GC) with flame ionization detection using a Hewlett–Packard 6890 series gas chromatograph provided with an auto-sampler. The chromatographic conditions were identical to those of the GC–MS analysis using air and hydrogen at flow rates of 400 and 40 ml/min, respectively. The automatic electronic integration of data with the software HP Chemstation Version B.01.00 was used to quantify the percent area of a sample.

2.3. Insect rearing

The adult *A. ludens* were kindly provided by the insectary of the Institute of Biotechnology at the Universidad Autónoma de Nuevo León (UANL) in Nuevo León, México. The flies were maintained under controlled conditions at 25 °C with a 16:8 h light:dark photoperiod and were fed the Shorey solid diet (Shorey and Hale, 1965) and a solution of 10% sucrose.

2.4. Ingestion toxicity assessment

The bioassays were performed with 20 adult flies (two-day-old, 10 males and 10 females) placed in a transparent one l plastic cup with a mesh fabric covering the top (Buentello-Wong et al., 2015), The flies were fed ad libitum with different dilutions of the three essential oils [for the EO of *E. caryophyllus*, the following concentrations (w/v) were tested: 0.05, 0.2, 0.3, 0.5, 0.75, 0.8, 1.2 and 1.5%. For the EO of *O. basilicum*, the following concentrations (w/v) were tested: 0.2, 0.5, 0.75, 0.8, 1.2, 1.5, 2.45 and 3.5%. For the EO of *T. vulgaris*, the following concentrations (w/v) were tested: 0.08, 0.2, 0.5, 0.75, 0.8, 1.2, 1.5 and 2%]. The dilutions were prepared with a solution of 10% w/v sucrose plus 1% w/v Tween 20 to ensure oil emulsion and were placed in a 24 ml plastic cup. The cup had a hole in the lid filled with a piece of cotton, which allowed the flies to feed on the emulsion through capillary action without drowning. The flies were also provided 20 g of Shorey solid diet (Shorey and Hale, 1965). Mortality was recorded daily for seven days. Three replicates per assay were conducted, including a control with only 10% w/v sucrose plus 1% w/v Tween 20 and 20 g of Shorey solid diet.

2.5. Statistical analyses

The mortality values were corrected with Abbott's formula (1925) when mortality was observed in the control group. The differences among mortality for the essential oils were determined by one-way analysis of variance (ANOVA), and means were separated by Tukey–Kramer tests (Sokal and Rohlf, 1981); a probability level of $P < 0.05$ was used to determine significant differences among means. The data obtained from each dose–response bioassay were subjected to probit analysis, and the LC50 and LC90 values and 95% confidence intervals were estimated. The comparisons of LC50 or LC90 values were based on the overlap of confidence intervals (Finney, 1978). All analyses were conducted using the SPSS 14.0 statistical software package (SPSS Inc., Chicago, IL, USA 2004).

3. Results

3.1. Analyses of essential oils

A total of 30 components were identified in the three EOs, which accounted for 96–97% of the total composition (Table 1). The primary components of the EO of *E. caryophyllus* were eugenol (77.58%), acetyl eugenol (10.99%), β-caryophyllene (6.22%) and α-humulene (0.90%). For *T. vulgaris*, the primary EO components were thymol (36.85%), *p*-cymene (32.49%), α-terpineol (12.58%), linalool (5.29%) and α-pinene (2.18%). For *O. basilicum*, the primary components were estragole (72.64%), linalool (16.65%), *cis*-α-bisabolene (1.48%) and neral (1.41%). The EOs of *E. caryophyllus* and *O. basilicum* were composed largely of phenylpropanoids (77.58% and 72.63%, respectively), which were followed by benzoate esters (10.99%) and sesquiterpenes (6.22%) in *E. caryophyllus* and monoterpenes (16.65%) in *O. basilicum*. For *T. vulgaris*, the EO was primarily composed of monoterpene hydrocarbons (89.39%).

3.2. Ingestion toxicity

The mortality of adult *A. ludens* was linearly correlated with the concentration of ingested EOs (Fig. 1). The mortality over 50% occurred on day three at 0.5% (w/v) for the EO of *E. caryophyllus*, on day five at 1.5% (w/v) for the EO of *T. vulgaris*, and on day seven at 1.2% (w/v) for the EO of *O. basilicum* (Table 2). The highest concentration of the EOs was 1.5, 2 and 3.5% w/v for *E. caryophyllus, T. vulgaris* and *O. basilicum*, respectively, and these concentrations caused 100% mortality on day 3, 7 and 7, respectively (Fig. 1). The mortality in the controls was 2%. Ordered from high to low toxicity, the LC50 values for *E. caryophyllus*, *T. vulgaris* and *O. basilicum* were 3529, 5347 and 8050 ppm, respectively; all were significantly different, and the LC90 values were 7763, 18,113 and 25,846 ppm, respectively (Table 3).

4. Discussion

4.1. Analyses of the components of essential oils

In this study, the primary components of the EO of *E. caryophyllus* were similar to those reported by others (Fichi et al., 2007), with slight variations in the percentages of eugenol (77.58%) and