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Essential oils from *Dysphania ambrosioides* and *Tagetes minuta* enhance the toxicity of a conventional insecticide against *Alphitobius diaperinus*

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ABSTRACT

The darkling beetle *Alphitobius diaperinus* is one of the most common pests in poultry farms, with its occurrence causing several problems including the dispersion of pathogenic microorganisms, injuries and stress in birds, as well as structural damage to the facilities. The aim of this study was to investigate the chemical composition of essential oils (EOs) from *Dysphania ambrosioides* and *Tagetes minuta*, and to determine their contact toxicity alone and in combination with cypermethrin against adults of *A. diaperinus*. The main components of the EOs were ascaridole, *p*-cymene and carvacrol in *D. ambrosioides* oil, and dihydrotagetone, *cis*-ocimenone, *trans*-tagetone and *trans*-β-ocimene in *T. minuta* oil. The EOs from both plants showed a high contact activity, while cypermethrin was slightly toxic to the insect when applied alone. The toxicity of *D. ambrosioides* oil was six times better than that of *T. minuta* oil, and more than fifty times more effective than cypermethrin. When cypermethrin was applied in combination with the EOs at low concentrations, the toxicity of this insecticide increased significantly. As the EOs studied have interesting properties against *A. diaperinus*, their use could be considered in new strategies for pest management.

1. Introduction

The darkling beetle *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) is one of the most common pests in poultry farms. Its massive occurrence decreases productivity and causes economic losses and health problems for both birds and humans (Schroeckenstein et al., 1988). This insect acts as a vector and competent reservoir of several pathogens, such as viruses (Ou et al., 2012) and bacteria including *Bacillus* sp., *Campylobacter* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* sp., and *Staphylococcus* sp. (Chernaki-Leffer et al., 2002; Hazeleger et al., 2008; Agabou and Alloui, 2010), thereby favouring the dispersion of microorganisms in poultry houses (Leffer et al., 2009). At the same time, it directly affects birds due to the fact that both adults and larvae may cause skin lesions (Uemura et al., 2008). Furthermore, the intake of larvae instead of balanced feed affects chicken growth (Despins and Axtell, 1995), with the birds exposed to this insect showing signs of stress (Crippen and Esquivel, 2012). In addition, this coleopteran damages materials used in constructing the facilities, especially when larvae burrow into the polystyrene and polyurethane to pupate (Despins et al., 1991).

The most common method to control this pest is the use of synthetic insecticides, mainly pyrethroids and organophosphates (Szczepanik et al., 2008). These compounds are applied by spraying the floor and walls before the replacement of the litter for the next breeding cycle to avoid direct contact with birds (Salin et al., 2003). However, due to the excessive use of these insecticides, a loss of field efficacy and the development of resistant insect populations have been reported (Chernaki-Leffer et al., 2011; Lambkin and Furlong, 2011). Consequently, there has been an increased interest in alternative management practices, including the use of botanical products such as essential oils (EOs) (Szołyga et al., 2014; Wang et al., 2014), which are complex mixtures of volatile secondary metabolites produced by aromatic plants

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(Bakkali et al., 2008). These are considered to be interesting alternatives to synthetic insecticides because of their limited persistence in the environment, low probability of generation of resistance (Isman, 2000; Koul et al., 2008), and low toxicity for vertebrates (Hummelbrunner and Isman, 2001).

The effects of EOs on survival, development, behaviour and reproduction of different insect species have been widely documented (e.g. Koul et al., 2008; Ebadollahi and Jalali Sendi, 2015). In particular, EOs from Alliaceae, Asteraceae, Cupressaceae, Lauraceae, Myrtaceae, Poaceae and Rutaceae have shown fumigant, contact, repellent and/or antifeedant activities against larvae and adults of *A. diaperinus* (Pinto Junior et al., 2010; Szczepanik et al., 2012; Gonçalves Marques et al., 2013; Szołyga et al., 2014; Wang et al., 2014). There is also some evidence that EOs, when applied in sublethal doses, synergize the activity of synthetic insecticides against aphids (Faraone et al., 2015), lepidopterans (Fazolin et al., 2016) and mosquitoes (Tong and Bloomquist, 2013; Gross et al., 2017). Thus, using EOs could enable a reduction in the amount of insecticides required in pest management plans and consequently in the undesired effects generated by their use.

Dysphania ambrosioides (L.) Mosyakin & Clemants (Chenopodiaceae) and *Tagetes minuta* L. (Asteraceae) are native plants of South America and are currently distributed throughout several tropical, subtropical and temperate regions of the world, with the EOs from these plants having proven insecticidal activity against different species of insects (Elidrissi et al., 2014; Nenaah et al., 2015; Pavela et al., 2017). The aim of this study was to determine the chemical composition of EOs from *D. ambrosioides* and *T. minuta* and their contact toxicity on adults of *A. diaperinus*. In addition, the effect of a conventional cypermethrin-based insecticide alone and in combination with EOs was evaluated. The EOs were expected to be toxic on their own and to increase the toxicity of the insecticide used in combination against *A. diaperinus*. The use of natural products together with conventional insecticides may enable the amount of xenobiotics needed to control this pest to be reduced.

2. Materials and methods

2.1. Test insects

All experiments were performed using adults of *A. diaperinus* obtained from Colonia Caseros, Entre Ríos, Argentina. Insects were placed in plastic containers with the same litter used for birds on the farm and provided with poultry feed. They were maintained in the laboratory under controlled temperature and relative humidity (28 °C and 70%), and unsexed adults of mixed ages were used for the bioassays.

2.2. Essential oil extraction and analysis

The EOs were obtained from fresh leaves (200 g) of *D. ambrosioides* and *T. minuta* (the plants were harvested at full fruiting) by steam distillation for 2 h in a glass Clevenger-type apparatus and stored at −20 °C in air tight microtubes prior to analysis by gas chromatography-mass spectrometry (GC–MS). The oil yields were 1% and 1.5% (w/w), respectively.

The EOs were studied using the following two analytical systems; i) GC analysis was carried out using a Perkin Elmer 500 equipped with a FID and a DB-5 capillary column (30 m × 0.25 mm i.d. and 0.25 μm film thickness). The initial oven temperature was 60 °C for 5 min, and then increased from 60 °C to 250 °C at 5 °C/minute, with a final hold time of 10 min; injector temperature, 250 °C; detector temperature, 280 °C; carrier gas, 1.0 mL/min nitrogen. ii) GC–MS analysis was performed using a Perkin-Elmer 600-SQ8 GC–MS system coupled with a quadrupole analyser and the same capillary GC conditions as described above. A 2 μL sample was manually injected with a 1:100 split ratio. Helium was used as the carrier gas with a flow rate of 0.9 mL/minute, and ionization was performed by electron impact at 70 eV. Mass spectral data were acquired in the scan mode in the *m/z* range 35–450. Retention indices (RI) of the sample components were determined on the basis of homologous *n*-alkane standard hydrocarbons (series C_6-C_{18} (ICN biochemical Co.) under the same conditions and the standard hydrocarbons were of analytical grade). The compounds were identified by comparing their retention indices and mass spectra with previously published data (Adams, 1995) and NIST and Adams libraries. The main components were further identified by coinjection of authentic standards (Sigma-Aldrich, USA), and fenchone was used as the internal standard at a concentration of 0.1 mg/mL dichloromethane. The quantitative composition was obtained by peak area normalization, and the response factor for each component was considered to be equal to 1.

2.3. Chemicals

Commercial grade cypermethrin 5% (Vetancid®, Vetanco S.A., Buenos Aires, Argentina), a pyrethroid insecticide commonly applied in poultry farms, was used in the bioassays. The recommended concentration indicated is 5 g/m^2. Piperonyl butoxide (PBO) 90% technical grade was obtained from Sigma-Aldrich (Buenos Aires, Argentina). The following pure compounds were purchased: D-limonene (code CRM40422, Sigma-Aldrich, solution ≥99.5% (GC)), *p*-cymene (code 30039, Sigma-Aldrich, solution ≥99.5% (GC)), and β-*trans*-ocimene (code W353901, Sigma-Aldrich, solution ≥90% (GC)). Piperitenone epoxide, a solution mixture of isomers cis/trans ≥95% (GC)), was provided by Prof. Abburra, Universidad Nacional de Córdoba, Chemical Engineering School. *trans*-tagetone, *cis*-ocimenone, and trans-β-ocimenone were obtained by supercritical fluid (Herrera et al., 2015).

2.4. Contact toxicity assay

The insecticidal activity of the EOs against adults of *A. diaperinus* was evaluated using a contact toxicity assay described by Herrera et al. (2015). A series of dilutions of the EOs or cypermethrin 5% were prepared in acetone, and then 200 μL of each dilution were applied to filter paper disks placed in glass Petri dishes (6 cm diameter). *Dysphania ambrosioides* oil was tested at concentrations of 4.5, 9, 20, 25, 30, 40, and 50 μg/cm^2. *Tagetes minuta* oil was tested at 50, 100, 150, 200, and 282 μg/cm^2. Cypermethrin 5% was tested at 100, 300, 500, 750, and 900 μg/cm^2. The solvent was allowed to evaporate for 2 min prior to the introduction of ten adult insects in each Petri dish. Control insects were kept under the same conditions but with only acetone. All treatments were replicated five times and mortality was checked after 24 h. Insects were considered dead when they showed no movement when touched with tweezers.

2.5. Joint action assay

Bioassays were performed in order to evaluate if the EOs extracted from *D. ambrosioides* and *T. minuta* increased the susceptibility of *A. diaperinus* to cypermethrin. The combination of cypermethrin with PBO, a well-known synergist of pyrethroids (Bernard and Philogene, 1993), was used as positive control. Different concentrations of cypermethrin (100, 300, 500, 750, and 900 μg/cm^2) were mixed with fixed amounts of the EOs or PBO. In the mixtures, the EOs were used at LC_{25}, while PBO was used at 900 μg/cm^2 since it was not toxic for *A. diaperinus* at that concentration. Experiments were carried out following the same method used for the contact toxicity assay described in section 2.4, with mortality being registered at 24 h.

2.6. Statistical analysis

The concentration-mortality data were subjected to a Probit analysis to determine lethal concentrations (those causing 25 and 50% of mortality (LC_{25} and LC_{50})), as well as their confidence limits at 95%. The values of LC were considered to be significantly different if their