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Insecticidal activity of microencapsulated *Schinus molle* essential oil

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ABSTRACT

Microencapsulation of the *Schinus molle* Rev L. (Anacardiaceae) leaves essential oil (EO) has been employed to control the release of active ingredients, protecting them from the external environment, with the concurrent improvement of its insecticidal potential on *Haematobia irritans*. Four microcapsule formulations (EEO1 to EEO4) of *S. molle* EO were prepared by spray-drying, using a mini spray dryer and gum Arabic/maltodextrin (AG/MDX) as the carrier in different proportions, at a ratio of 4:1 (MDX/AG:EO). Encapsulation efficiency (EE: 96–100%), powder morphology and particle size distribution were analyzed as responses. An interesting correlation was found between EO free and microcapsules (EEO) in the preliminary and comparative studies of stability (at 45 °C) and in the insecticide activity on *H. irritans*. In fact, a very slow liberation profile of the microencapsulated EO (EEO4) was observed over a period of 366 h (71% of EO retention), as well as a slower time-dependent insecticide effect (32 and 73% of dead flies at 2 and 4 h of exposure time) compared to the free EO (96% of dead flies at 2 h).

1. Introduction

The horn fly, *Haematobia irritans* (L.) (Diptera: Muscidae), is one of the most important blood-sucking pests of pastured cattle, causing annual economic losses estimated at 1 billion and 150 million dollars in the U.S. and Brazil respectively (Barros et al., 2001; Cupp et al., 2004; Guglielmone et al., 1999, 2001; Oyarzún et al., 2008). This may result in a decreased milk production, reduced weight gains and poor feeding efficiency. Consequently, livestock producers must select an appropriate control method to manage this pest.

The control method used initially was chemical insecticides, but this strategy has led to resistance to most commercially available products (Guglielmone et al., 1999, 2001; Barros et al., 2001). Additionally, the use of those chemicals has negative consequences, such as soil and water contamination, high persistence in the environment, intoxication of people and animals, and residues in foods (Regnault-Roger et al., 2004).

Consequently, the search for new pest controls such as botanical insecticides appears as a more attractive ecological and natural alternative to be explored as integrated pest control.

Schinus molle (L.) (Anacardiaceae) is an highly aromatic evergreen tree native to South America, whose volatile oils have been thoroughly studied (Murray et al., 2005; Rossini et al., 1996), and to which different biological activities are attributed (antibacterial, anti-fungal, anti-inflammatory, cytotoxic, insecticidal) (Atti dos Santos et al., 2009; Abdel-Sattar et al., 2009, 2010). Interestingly, most of these activities correspond to the essential oils protective role the plants have in nature (Bakkali et al., 2008). Specifically, some studies related to the insecticidal and repellent effects of *S. molle* extracts against different insects have been published (Ferrero et al., 2006, 2007; Abdel-Sattar et al., 2009; Deveci et al., 2010).

However, the biological activity of these products can be lost through the volatilization of their components or their degradation (exposure to high temperatures, oxidation and/or UV radiation), making the commercial applications of these oils limited. What is more, essential oils have short residual activity, which reduces the workers' exposure to residues, but this may result in repellent activity and the need for repeated applications in order to obtain insecticidal activity, which may lead to higher phytotoxicity risks (Cloyd and Chiasson, 2007).

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Besides, as an alternative for specific applications, volatile oils can be prepared in a large number of formulations: liquid, semi-liquid or solid forms to be used to control the release of active ingredients and to protect them from the external environment (Miró et al., 2010).

Microencapsulation is one of the most efficient processes for this kind of products. Basically, microencapsulation technology consists in "packing" an active ingredient within a wall material, thus transforming an emulsion into a more stable powder (Tonon et al., 2011). The release of the functional agent occurs by diffusion through the capsule wall and/or rupture of the microcapsules. Therefore, the use of microcapsules could provide a durable repellent and/or insecticidal finish (Miró et al., 2010). At present, different techniques are used for the microencapsulation of food additives (flavors, preservatives, leavening agents, vitamins and minerals). Spray-drying is a low-cost microencapsulation technology commonly used in the industry, which has the attractive advantage of producing microcapsules in a relatively simple, inexpensive and continuous operation, compared to other conventional microencapsulation techniques. Moreover, from a commercial point of view, it has been estimated that the cost of spray-drying is six times lower, per kilogram of water removed, than the cost of freeze-drying (Knorr, 1998). What is more, this technique has been widely used for drying heat-sensitive materials (foods, pharmaceuticals) because of the rapid evaporation of the applied solvent from the droplets (Kolanowski et al., 2005).

Spray-drying involves the atomization of emulsions into a drying medium at a high temperature, which leads to very fast water evaporation. This results in quick crust formation and quasi-instantaneous entrapment of the core material (Gharsallaoui et al., 2007; Tonon et al., 2011; Pulido and Beristain, 2010). Some of the advantages of this method are its ability to handle heat-sensitive materials, the availability and diversity of equipment, the variety of particle sizes produced and an excellent dispensability of particles in aqueous media. Conversely, the main disadvantages of this technology are the high temperature and air exposure necessary for the drying process. Moreover, the product can adhere to the surface of the capsules during drying, thus causing potential modifications of the end product formulation (Kanawija et al., 1992). Spray-drying technology also requires well-adjusted operating conditions, as well as the correct composition of the solution that contains the active principles (Vaidya et al., 2006; Gallo et al., 2011; Soliman et al., 2013).

In this work we describe the preparation and characterization of microcapsules of *S. molle* essential oil (EEO) using the spray-drying technology. Operational parameters such as concentration of wall material on loading capacity and encapsulation efficiency (EE) were studied. The microcapsules were evaluated for the content and stability of EO, and scanning electron microscopy was used to observe the powder morphology and particle size distribution. Insecticide activity was also studied using the *H. irritans* bioassay model. The experimental model developed should provide valuable insight in order to correlate the insecticide efficacy of the microencapsulated essential oil with storage and application times.

2. Materials and methods

2.1. Chemicals

Laboratorio Uruguay S.A. (LUSA, Montevideo, Uruguay) kindly donated technical-grade diazinon (87.9% AI). Gum Arabic and maltodextrin were purchased from Parafarm (Saporiti, Argentina).

2.2. Plant material and isolation of the essential oil

The selection of the natural active material used (*S. molle* essential oils, EO) was carried out by means of an in vitro screening of insecticide activity by using the bioassay previously developed and reported against *H. irritans* (Andina et al., 2012). Samples of fresh leaves and stems, representing the entire population of *S. molle*, were collected randomly at the Centro de Extensión y Capacitación en Plantas Aromáticas y Medicinales (Monte Vera, Santa Fe, Argentina) during the full flowering period (September to November). The samples were representative of the species and were chosen in order to be representative of the same pedoclimatic and collection conditions; the extraction conditions were also identical for all samples. The essential oil was obtained from fresh leaves and stems by classical steam distillation for 2 h at normal atmospheric pressure in pilot-scale stainless steel equipment.

2.3. Analysis and identification of EOs

The composition of the oil was determined by GC using a Shimadzu (Tokyo, Japan) model 14 B gas chromatograph equipped with a FID and Shimadzu EZ-Chrom data-processing software. Analyses were conducted following the IRAM-ISO/TC 54 Norm, with minor modifications. Chromatography was performed using a DB-WAX (Agilent J&W, USA) fused-silica capillary column (30 m × 0.250 mm i.d.), coated with polyethylene glycol (0.25 μm phase thickness); the oven temperature program was 40 °C for 8 min, raised to 190 °C at 15 °C/min, and then to 230 °C at 30 °C/min, finally maintained at 230 °C for 20 min; the temperature of the injector was 210 °C; the temperature of the flame ionization detector (FID) was 230 °C; the gas carrier was nitrogen (100 kPa); the injection mode was split with a split ratio of 1:40.

The components of the oil were identified by comparing their linear retention indices (LRIs) to those of pure standards or as reported in the literature (Atti dos Santos et al., 2009). The percentages of each component were reported as raw percentages without standardization. Repeatability of the measuring system showed variation coefficients under 5% for all the components.

2.4. Preparation of microcapsules by spray-drying

2.4.1. Preparation of emulsions

Four different suspensions (1–4) were prepared using maltodextrin (MDX) and gum Arabic (AG) as carrier (wall material) in different proportions (4:1, 3:2; 2:3; and 1:1, respectively). MDX and AG were previously dissolved in distilled water at 50 °C for 2 h and left to stand for 12 h at room temperature. For the emulsion preparations, the EO of *S. molle* was incorporated into the wall material suspension using an Ultraturrax T18 homogenizer (IKA(R) T18 basic, Staufen, Germany) at 24,000 rpm for 30 min. The emulsions obtained (50–60 mL) were stored at room temperature until use, and were examined with optical light microscopy applying 100× magnification using an Olympus microscope (BX41, Tokyo, Japan).

2.4.2. Spray-drying

Spray-drying was performed using a laboratory-scale Mini Spray Dryer (Büchi B-290, Büchi Labortechnik AG, Flawil, Switzerland). The samples were atomized with a hot air stream in the drying chamber, thus making it possible to obtain solid microparticles where the EO was trapped within a film of encapsulating material. A two-fluid nozzle of 0.5 mm cap orifice diameter was used. The following parameters were fixed: pump, 15%; aspirator %, 100; Q-flow, 600 l/h; inlet temperature, 160 °C; outlet temperature, 100 °C.