International Biodeterioration & Biodegradation 116 (2017) 227–233

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Microencapsulation of *Lippia turbinata* essential oil and its impact on peanut seed quality preservation

Natalia S. Girardi [a, b], Daiana García [a, b], María A. Passone [a, b, *], Andrea Nesci [a, b], Miriam Etcheverry [a, b]

[a] Laboratory of Microbial Ecology, Department of Microbiology and Immunology, National University of Río Cuarto, Ruta Nac. 36, km 601, Río Cuarto, Argentina
[b] National Council of Scientific and Technical Researches (CONICET), Argentina

ARTICLE INFO

Article history:
Received 10 December 2015
Received in revised form
2 November 2016
Accepted 2 November 2016

Keywords:
Poleo essential oil
Peanut
Microencapsulation
Fungal pathogens
Seed germination

ABSTRACT

In this study the use of complex coacervation to encapsulate the essential oil (EO) of *Lippia turbinata* (poleo) and its application for controlling fungal pathogens of peanut seeds were evaluated. High percentages of encapsulation of poleo EO were obtained (99.80%). At the end of storage period (78 d), between 3.5 and 63% of poleo EO were released from microcapsules, this action was favoured by the increase of grain a_W. The formulation of poleo EO showed a significant antifungal effect on peanut mycoflora, with reductions between 59 and 77%. Mycological studies showed a prevalence of *Penicillium* and *Aspergillus* lesser extent in peanut seeds throughout the storage period. The formulation caused complete inhibition of peanut seeds germination. In conclusion, the gelatin/gum arabic system was effective in encapsulating poleo EO by allowing its controlled release. Microencapsulated poleo EO maintained the antifungal activity, but produced allelopathic effects on peanut seeds germination.

1. Introduction

Peanut (*Arachis hypogaea* L.) is an economically important crop throughout the world. Argentina is the sixth producer of peanut with 3.0% of overall world production, ranking first in the category of peanut exporters worldwide (USDA, 2015). Córdoba province is responsible for 93% of the national peanut production with a cultivate area of 345,200 ha (BCCBA, 2015). Nevertheless, this crop is susceptible to many pathogens, with most damage caused by fungi. Soil-borne fungal diseases adversely affect peanut health and productivity all over the world growing areas. Depending on severity of field infestation, yield losses due to such soil-borne disease may be as high as 50%. The diseases are caused by seed borne pathogens that can survive in infected peanut seeds (Melouk and Backman, 1995). Therefore, harvested peanut seeds contain fungal mycelia and spores that can result in a significant decrease in seed quality when they are stored. This is an important aspect, considering that in our country producers preserve peanut seeds for the next planting harvested over a period of 4–6 months. During storage, a microorganism's succession is development on the grains, which is determined by physical–chemical (moisture, temperature, pH, levels of O_2, chemical additives and storage time) and biological conditions (interactions with other microorganisms, presence of insects and rodents) that allow transitory or permanent changes in the fungal population (Lacey and Magan, 1991). The most common fungi identified from peanut seeds stored in different storage systems included *Penicillium*, *Aspergillus*, *Eurotium* and *Fusarium* spp. Within *Aspergillus* genus, the section *Flavi* had the greatest mean counts of 1.4×10^4, 9.4×10^2, 5.2×10^2 cfu g^{-1} for big bags, wagon and warehouse, respectively (Passone et al., 2014). Consequently, storage fungal infection can reduce the quality and the seed viability. Seeds contaminated with pathogenic fungi can act also as a source of inoculum for diseases by affecting the crop during its development, or as a vehicle for dispersion through which different pathogens can be introduced in a field or region. Therefore, the quality of the seeds is essential to establish an appropriate stand of plants for the production of peanut crop (Ketring, 1991).

Usually the germination and seed vigor tests are evaluated, but their sanitary quality is generally not considered, assuming sufficient conduct a seed fungicide treatment (March et al., 2003).

* Corresponding author. Laboratory of Microbial Ecology, Department of Microbiology and Immunology, National University of Río Cuarto, Ruta Nac. 36, km 601, Río Cuarto, Argentina.
E-mail address: apassone@exa.unrc.edu.ar (M.A. Passone).

http://dx.doi.org/10.1016/j.ibiod.2016.11.003
0964-8305/

Effective control of root and stem wilt diseases can be reached by supplying a recommended fungicide. Thus, seed treatments with chemical fungicides, such as thiophanate methyl [dimetil-4,4'-(o-fenileno) *bis* (3-tio alofanato)] and metalaxyl [metil D,L-*N*-(2,6 dimetil fenil)-*N*-(2-metioxi-acetil)-alaninato] are frequently used in combined form to protect the seed from pathogenic organisms before sowing. Both agrochemicals are systemic fungicides that play a very important role in plant disease control and they are applied world-wide on numerous crops. The fungicidal activities mechanism of thiophanate metyl (carbamate group) is due to the cholinesterase inhibitory activity, while metalaxyl (derivative of acilanines) causes protein synthesis inhibition (Leroux, 2002). This strategy could contribute to environmental pollution added to the hazard that it normal use represent on human health (Silva and Fay, 2006; Pires et al., 2005). Therefore, there is a considerable interest in finding alternatives to synthetic chemical pesticides for suppression of soil-borne plant pathogens (Haggag, 2007).

The use of natural compounds offers an alternative in replacing synthetic chemicals intended to plant diseases control. Essential oil (EO) of *Lippia turbinata* (poleo) has proven *in vitro* effectiveness for controlling *Aspergillus* on culture medium and on irradiated peanut kernels by contact (Passone et al., 2012a; b) and vapor tests (Passone et al., 2013; Passone and Etcheverry, 2014). However, EO levels quickly decreased when it was applied in the peanut food systems. Microencapsulation technology is one of the most effective methods to date to achieve controlled release of the compounds (Moretti et al., 2004; Hussain and Maji, 2008). This process creates a physical barrier between the core and wall materials protecting sensitive ingredients from the external environment, particularly moisture, pH and oxidation (Nesterenko et al., 2013).

Thus, the aims of this work were: i) to encapsulate poleo oil by complex coacervation method; ii) to evaluate the encapsulation parameters; iii) to analyze the microcapsule application for controlling fungal pathogens in peanut intended for seed; and iv) to study the effect of microencapsulated poleo oil on peanut seed germination.

2. Materials and methods

2.1. Collection and characterization of the essential oil

Dried leaves of *Lippia turbinata* (poleo) were purchased from a local market. The identification was done according to Cantero and Bianco (1986). The plant material was stored at 4 °C after harvest. A portion (100 g) was submitted for 3 h to water-distillation, using an extractor of essential oils (EOs) by steam distillation at laboratory scale (Figmay S.R.L.) (yield 1.02%). The obtained EO was dried over anhydrous sodium sulfate and, after filtration, stored in a sterilized vial at 4 °C. The characterization of poleo EO was performed by gas chromatography mass spectrometer (GC/MS) (Passone and Etcheverry, 2014).

2.2. Preparation of coacervate microcapsules

Poleo EO was used as core material, while gelatin (type A, gel strength 240 bloom) and gum arabic were used as the wall material. The microcapsules were made by complex coacervation adapting the methodology proposed by Girardi et al. (2015).

Twenty five mL of gelatin and gum arabic solutions 5% w/v were prepared at 50 °C in a thermostatic bath (Decalab SRL). pH of gum arabic solution was adjust to 6 with sodium hydroxide 1M (NaOH). Poleo EO (450 μL) was added into the gum arabic solution, forming an emulsion by magnetic stirring (Auto Science, AM-5250B). Then, gelatin solution was added and the mix was stirred at 400 rpm during 10 min at 50 °C. After that, pH was adjusted to 4 with hydrochloric acid 1M (HCl) and the stirring was continued for 10 min. Subsequently, pH was adjusted to 9 with NaOH 1M and stirred for 10 min. After that, the temperature was lowered to 10 °C in an ice bath and 5 mL of formaldehyde was then added to compact the gelatin/gum arabic coating. Crosslinking time was 10 min at room temperature.

Finally, microcapsules were washed twice with distilled water and were stored at −20 °C until the lyophilization step. For lyophilization process, microcapsules were previously frozen at −80 °C during 3 h (L-T8-A-B3-CT, RIFICOR). Then the samples were ground (CT 193 Cyclotec™ Sample Mill) to obtain a fine powder (1000 μm).

2.3. Efficiency of encapsulation

Efficiency estimation was adapted from Kaushik and Roos (2007). Microcapsules (0.5 g) were added to 10 mL of chloroform (HPLC Grade, Sigma Aldrich) in glass flask and shaken in an orbital shaker for 5 min. Powder particles were separated from chloroformic extract by filtration. The test was performed in triplicate.

The amount of EO present on the surface of the microcapsules was estimated from chloroformic extracts by gas chromatography mass spectrometer (GC/MS Clarus 600, Perkin Elmer) equipped with a DB5 column (60 m, 0.25 mm ID, 0.25 μm particle Perkin Elmer). To control the equipment and data acquisition the Turbo Mass program was used. Working conditions were: initial temperature 60 °C (5 min) ramp: 5 °C/min, final temperature 115 °C. The mobile phase used was Helio 5.0 to 49.6 psi. The temperature of the injector and GC transfer line was maintained at 250 to 200 °C, respectively. Ionization was performed in the mass spectrometer vacuum with electron impact ionization energy −70 eV. The injection volume was 1 μl. The chromatogram was obtained in 'scan' mode from m/z = 50 to m/z = 350 (scan time 0.2 s, inter-scan time: 0.1s). The identification of the components of the poleo EO was performed by comparison with spectra libraries NIST MS Search 2.0.

For quantification of the residual oil, an external calibration curve of limonene (MW: 136.23 g/mol; CAS: 5898-27-2) was used, due it was the main component of pure poleo EO. The quantification curve in the range of sample concentrations (0.008232−1.0976 μg/μL of limonene, $R^2 = 0.9626$) was performed. Each concentration level of standard solution was analyzed by GC/MS in triplicate. Quantification was performed by reporting the measured integration areas in the calibration equation of the corresponding standards. The detection (LOD) and quantification (LOQ) limits of the analytical method for limonene 0.02 and 0.82 ng/g were 0.05 and 0.30 ng/g.

The encapsulated poleo oil was determined as the difference between the theoretical initial level and values estimated on the microcapsule surface. Efficiency encapsulation was expressed as the percentage of poleo oil encapsulated with respect to the initial amount.

2.4. Release property of poleo EO microcapsules

Release of microencapsulated poleo EO was evaluated *in vitro*. To characterize the formulation stability, 0.1 g of microcapsules were placed in flaks, which were then sealed and incubated at 25 and 4 °C. To evaluate the release of microencapsulated poleo oil on the substrate, peanut kernels were sterilized twice by autoclave at 120 °C for 20 min. Then water activity (a_W) was adjusted by aseptically adding sterile distilled water according to the calibration curve previously made. Sealed containers were stored at 4 °C for 48 h with periodic hand shaking during this time. Water activity values were confirmed with an AquaLab Series 3 (Series 4, TE, USA)