Original Article

Microencapsulation of essential oil of molle (Schinus Molle) against the aphid Macrosiphum euphorbiae

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Abstract

The essential oil of molle (Schinus molle) has proven biocidal activity, which can be enhanced by spray-drying. Therefore, an emulsion was prepared with this oil and chitosan (1:2) to be later micro-encapsulated with the Spray-drier YC 500 equipment. SEM morphologically evaluated the resulting powder and determined the amount of essential oil processed. Finally, a population of aphids (Macrosiphum euphorbiae) was dosed to determine the amount necessary to kill 50% of individuals (LD50) compared to the direct application of oil.

Keywords: Spray Drying; Schinus molle; Macrosiphum euphorbiae; Pest control.

Introduction

Microencapsulation is a protection process that allows solids, liquids or even gases to be enclosed in microscopic particles (from 1 to 1000 μm)(Singh et al. 2010). by the formation of thin shells or wall material around the substance of interest (Allen et al. 2005; Kishan et al. 2020).

In search of a substitute for paper and carbon, the commercial typewriter industry started this technique in the 1930s (Dhakal and He, 2020). The objective was to obtain gelatin dye capsules so that when the keys of a typewriter or pen were pressed against them, these capsules would release their contents. This was achieved in the 1950s, later, this technique was developed with other materials and other areas (Allen et al. 2005).

Microencapsulation technology has had varied applications, which are now increasingly diversifying. Despite its relatively high cost, multiple formulation steps and the low reproducibility of some techniques, microencapsulation technology has been used in various applications, which are now increasingly diversified (Dhakal and He, 2020).

Microencapsulation has been widely embraced and developed in the food industry, used to preserve valuable components and "enriching foods, while ensuring that the flavor, aroma or texture of the food is not compromised" (Sanguansri and Augustin, 2007).

Lau et al. (2017) and his team designed microcapsules for substances requiring protection against gastric digestion based on protein-tannic acid films that, when ingested, showed resistance to enzymes and the stomach environment but were vulnerable in the intestinal flow where they release their contents. Additionally, this product was complemented with immunoglobulins that allowed a better adhesion of these capsules to the intestinal epithelial cells, achieving a complete function.

On the other hand, microencapsulation is used in the textile industry "as a means of imparting finishes and properties on textiles that are not possible or cost-effective using other technology," such as fragrance finishes, flame retardant fabrics, polychromatic and thermochromic properties, among others (Nelson 2002), such as fragrance finishes, flame retardant fabrics, and polychromatic and thermochromic properties.

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In the medical area, microencapsulation presents itself as a practical solution to immunological problems, as demonstrated by Laporte et al. (2020) in recent research. The microcapsules with mesenchymal stem cells obtained offer the possibility of immunosuppressant-free therapy by physically isolating the islets from the immune system.

Finally, it is necessary to mention a sample of the development in the agro-industrial field and pest management, which the present research will reference.

Kavetsou et al. (2019), in his study, the encapsulation of Mentha pulegium essential oil as a biopesticide was used as encapsulating material biomass of the yeast Saccharomyces cerevisiae, concluded that "the encapsulated essential oil had a stronger insecticidal effect than the non-encapsulated oil 72 hrs after treatment".

The environmental approach that is taken when making this type of product, pollution and the growing resistance of pests to synthetic insecticides based on an active ingredient are factors that jeopardize the proper development of agribusinesses that each year will receive greater demand by the growing population rate.

Materials and methods

Materials and Equipment

The following materials were used in the study: Molle essential oil, Glacial acetic acid, Distilled water, Ethanol, N-hexane, Chitosan (SIGMA-ALDRICH), Tween 20, Magnetic stirrer with Hotplate MSM-20D-UNIT, Ultrasonic bath (MRC DC-150H digital ultrasonic cleaner), Microencapsulation equipment (Spray-drier YC 500), Spectrophotometer (UV-Visible Spectrophotometer GENESYS 150), Scanning Electron Microscope (ZEISS - EVO® MA 10), Glass Petri dishes, and Organza-type cloth (mesh).

Preparation of the emulsified solution

With a 1% acetic acid solution, 2% (w/w) chitosan was dissolved in a magnetic stirrer at 45°C until complete dissolution. Then, essential oil, equivalent to 1% (w/w) of the previous solution, was taken with Tween 20 at 7% (w/w concerning the oil), mixed and subsequently incorporated. The solution was then immersed in an ultrasonic bath for 10 minutes at 40°C.

Microencapsulation

The inlet solution was atomized using the Spray-drier YC 500. The inlet air temperature was set at 150°C and the air flow at 30 Hz. The resulting powder was collected and stored in amber bottles.

Morphological description of the microcapsules

Electron microscopy equipment (SEM) recorded the shape and possible structural variations and interactions in the microcapsules.

Determination of Total Encapsulated Oil

For this purpose, the following protocol was used (Li et al. 2013); 0.1 grams of microcapsules were taken was combined with 10 g of 1% acetic acid in a centrifuge tube for shaking until total dissolution. The released oil was extracted with h-hexane three times. The extracts were collected in a flask. The contents were then determined by measuring the absorbance in triplicate with a UV spectrophotometer.

Efficiency and Retention

Efficiency is the amount of product obtained about that expected due to losses or deficient parameters:

$$Efficiency\ (\%) = \frac{Processed\ volume}{Original\ Volume} * 100\%$$

Toxicity Tests

It was carried out with open glass plates, using aphids without distinction of developmental stages. A mesh covered the upper surface of each plate. Aphid behavior was observed during the tests, and mortality was recorded at intervals from 0 to 24 hours. This was replicated four times and control tests were carried out with empty microcapsules, adapted from (Moretti et al. 2002).

Statistical Analysis

The data obtained were analyzed with the corrected Abbott formula, the necessary statistical tests were performed with SPSS Statistic software (Carreras et al. 2009), and the necessary statistical tests were performed with SPSS Statistic software.

$$\%\ corrected\ mortality = \frac{\%\ mortality\ sample - \%\ mortality\ witness}{100 - \%\ mortality\ witness} 100$$

Results and discussion

1. Characterization of the morphology and size of the microcapsules

It can be observed (Image 1) that the obtained powder presents different particle sizes. In addition, it was possible to measure the dimensions of the most prominent microcapsules to check the microencapsulation process and evaluate the performance. The reference size corresponding to the molle essential oil microcapsules was 78.4 µm (Image 1). These dimensions are noteworthy because of the contrast with other works that reported sizes smaller than 20 µm. (Li et al. 2013; Vict et al. 2013; López et al. 2014; R.G. Kumar et al. 2017; Locali Pereira et al. 2019; Wirjosentono and Marpaung 2020)..

This phenomenon was due to a higher wall material ratio, in this case, 2:1 (chitosan: essential oil), the normal ratio being equal. (Li et al., 2013). However, this situation is better explained by higher emulsion stability and better oil retention per capsule, which would be corroborated later in aphid trials.

It is also necessary to consider the inlet temperature used (Kumar et al., 2017) found that by using a high value in this parameter, the particle size and the presence of folds on the surface of the capsules could be reduced; but in this situation, it would also increase the number of micropores that would put at risk the encapsulated material (Li et al. 2013; Vict et al. 2013).

On the other hand, the images show spherical shapes, wrinkled and concave surfaces or slight depressions; this is considered a typical phenomenon of microencapsulation (Lopez et al. 2014; R.G. Kumar et al. 2017) due to the rapid loss of water during the initial stages of drying. (Locali Pereira et al., 2019).

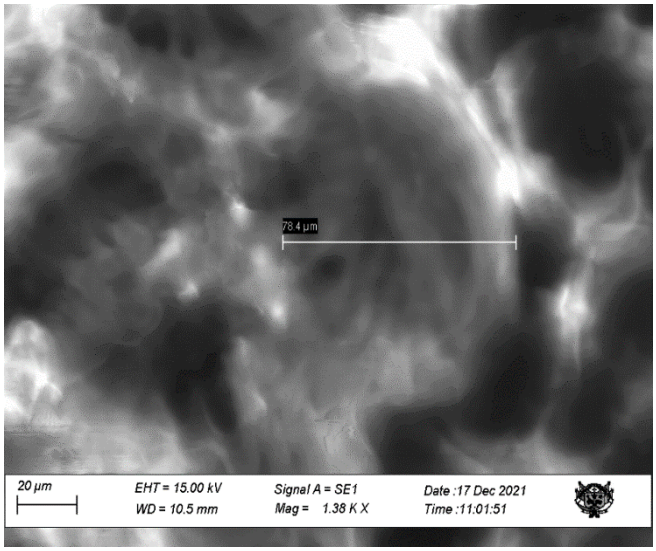


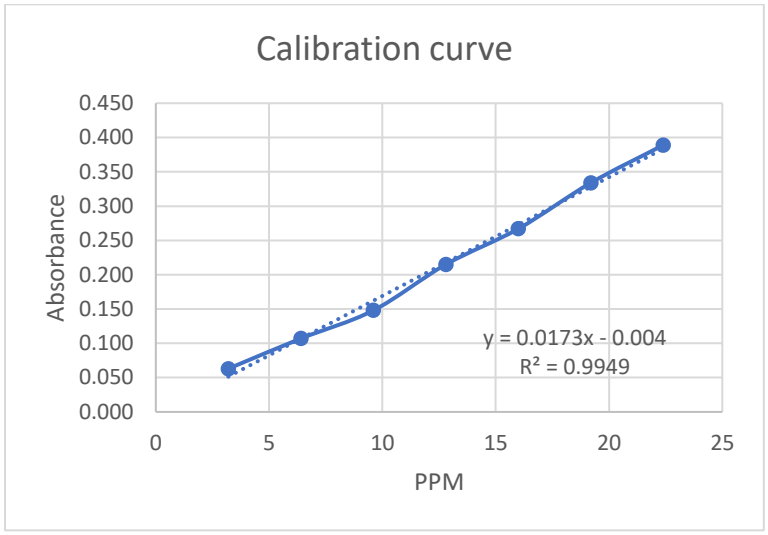
Image 1: Morphology of Schinus molle essential oil microcapsules by SEM.

1. Determination of Total Encapsulated Oil.

According to the procedure and the calculations performed (Annexes), the experiments were developed with a wavelength of 236 nm because they presented a marked peak and acceptable absorbance values. The results of the calibration curve are shown in Table X.

Table 1: Standard solutions with volume of 5 ml each, concentrations (ppm), absorbances and known masses.

Standard	PPM	ABS(236)	Milligrams
1	3.2	0.063	0.016
2	6.4	0.107	0.032
3	9.6	0.148	0.048
4	12.8	0.215	0.064
5	16	0.267	0.08
6	19.2	0.334	0.096
7	22.4	0.389	0.112



Graph 1: Calibration curve of standard solutions of Schinus molle essential oil. A correct progression and a trend line similar to the path of the points can be observed.

The absorbance obtained from the microencapsulated product was 0.086 and through the equation of the straight line of the trend line, it was known that the concentration of this sample was 5.34 ppm of essential oil of Schinus molle. The analyte is dissolved in a volume of 5 ml of n-hexane and determined that the real amount of essential oil was 0.026 mg.

2. Determination of Efficiency and Retention.

For efficiency, there was a noticeable loss of feed solution. This was always prepared to a final volume of 250 ml, including all reagents used.

At the beginning of the spray drying process, a quantity of solution slipped through the apparatus's hood and was deposited in a compartment whose purpose was to receive that residue. The portion was always similar (approximately 25 ml), so it could be assumed that 225 ml of feed solution were processed, equivalent to 90%.

This number may be due to several factors, including temperature and machine flow rate. However, the flow rate is a factory parameter that cannot be changed, and the temperature could not be higher than the one used as it would compromise the morphology and performance of the microcapsules protecting the oil.

3. Toxicity tests on Macrosiphum euphorbiae.

The information regarding the biocidal effect of the microcapsules was expressed in the percentage of mortality and later in LD50 (Lethal Dose Average). It is also necessary to mention that no dosage determined mortality values statistically different from the control group.

Table 2: Percentage mortality of each group of aphids in the different doses of microcapsules.

Dosage (mg)	**1**				**Mortality**
6.36	20	30	30	20	21.05
19.08	30	30	20	40	26.32
31.81	30	40	40	30	31.58
44.53	40	40	40	30	34.21
Witness	0	0	20	0	5

The maximum percentage of mortality was 40% at a dose of 44.53 mg. However, the LD50 could not be empirically determined so that it will be calculated according to the Profit analysis.

It is known that the essential oil of Schinus molle has repellent and insecticidal activity, as well as antibacterial and antibacterial action (Abdel-Sattar et al. 2010). In addition to antibacterial and antifungal action. The main components are β-Myrcene, α and β-Phellandrene, Cadinene, α-Pinene and Limonene (Bernhard et al. 1983; dos Santos Cavalcanti et al. 2015; Silva-Júnior et al. 2015). According to previous studies (Chambilla 2021), the essential oil used in this research contains D-limonene and α-phellandrene, which stand out for their biocidal activity and are irritant substances on contact.

It should be added that the microcapsules, being a fine powder, tended to become trapped in hair-like structures of the aphids' bodies, predominantly on legs and antennae. This situation could have enhanced the effect of the microcapsules.

Graph 2 shows the increase in mortality concerning the dosage of microcapsules. It can also be projected that the median lethal dose (LD50) is 87.19 mg of microcapsules in a Petri dish which could be converted to 1.3705 mg/cm^2.

When comparing the amount of microencapsulated essential oil that could correspond with an average lethal dose in this Petri dish system (87.19 mg), the amount of oil used in a similar-sized system by direct contact is 44.2982 mg (Chambilla 2021). It was found that the present work uses 1.3705 mg/cm^2whereas the previous one used 0.6964 mg/cm^2 to achieve the same objective. The difference in favor of the previous study is evident. However, since it is an essential oil without any covering or dosing system, it can be thought that to achieve the LD50 less quantity was required due to its volatile character that concentrated the environment of the evaluated population quickly. While the microcapsules have a controlled release that fills the environment gradually, they also provide greater protection to the oil and increase its useful life.

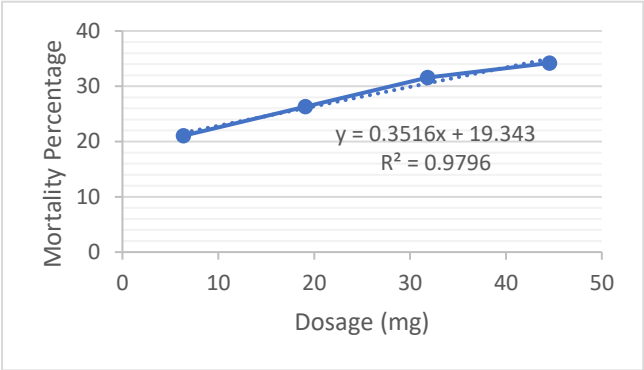


Figure 2. Mortality increases as the amount of microcapsules is fed to the aphid population after 24 hours of observation.

Conclusions

Microencapsulation of Schinus molle essential oil was possible and demonstrated that the unitary spray-drying operation did not impair its biocidal activity. It was also proved that the present study's microcapsules were larger than other studies due to a higher concentration of chitosan, which offers higher oil retention. On the other hand, no other disadvantage was detected by the morphology of these microcapsules.

The tests with aphids gave adequate results to determine the Mean Lethal Dose parameter that would allow homologating the biocidal effect to others offered by different substances. However, the threshold to reach the LD50 is higher than the one reached with the same oil by the direct contact method. The controlled release offered by microencapsulation may be the best explanation for understanding this difference.

CONTRIBUTION INFORMATION

Dr. Jesús María Zambrano Salas supervised and advised on the applied methodology and writing.

Magister Keny Davi Alvarado Quiroz gave his support in the realization and advice.

Luis Felipe Chambilla Miranda contributed with the experimental design, practical implementation and proofreading.

José Mauricio Conde Sánchez contributed to this work's experimentation, writing and proofreading.

CONFLICT OF INTEREST INFORMATION

The authors declare that they have no conflict of interest.

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ON RESEARCH ETHICS AND LEGAL STANDARDS

All established ethical standards were complied with.

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