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Development of formulation based on essential oils of rosemary to manage pests of stored cereal foodstuffs †

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Abstract: The aim of this work is to evaluate the insecticidal activity of the encapsulatedrosemary (*Rosmarinus officinalis* L.) essential oil coated intochitosan matrix. The effectiveness of crude and encapsulated oils has been studied during different storage periods (30, 45 and 60 days). Results revealed that the chitosan-essential oil formulation exhibited high insecticidal activity against adults of *Tribolium castaneum* as compared to crude essential oil during the different storage periods.

Keywords: Rosmarinus officinalis; Tribolium castaneum; Chitosan

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1. Introduction

Cereal production has been considered an important component of Tunisia agriculture [1]. Despite, the developpement efforts made by the authorities in this sector, Tunisian cereal still confronted to various problems [2] such as diseases [3] and insect pests [4]. Various species caused damage toward different stored products [5]. The use of chemical insecticides is one of the most known control methods for postharvest protection, due to its effectiveness [6]. However, previous researches indicated the undesirable effects of several chemical pesticides [7]. Thus, alternatives methods are well needed, which are more respectful to health and environment [8]. In this context, the valorization of plants extracts and essential oils [9] known by several bioactivities like fungicide, microbial and insecticides are required [10].

This work aims to provide innovative and appropriate methods for the control of insect pests of foodstuffs during storage, as well as, to restrict or eliminate the use of chemical insecticides.

2. Material and methods

2.1. Plant material

Rosmarinusofficinalis was collected from the arboretum Korbos (846 m 36° 48′54″ N 10 34′ 14″ E), north Tunisia. The collected samples were air-dried at ambient laboratory conditions.

2.2. Essential oil (EO) extraction

Essential oils were extracted by hydrostillation of 150 g of dried leaves using a modified Clevenger-type apparatus for 4 h as described by [11].

2.3. Insect rearing

T. castaneum was reared on semolina. The rearing conditions were 25 ± 1 °C, a relative humidity of 65 ± 5% and a photoperiod of 12h Light/12h dark. The boxes were observed

daily to collect adults according to their age group. Newly emerged adults (7 days old) were used for the bioassays.

2.4. Preparation of the Chitosan-essential oil formulation

The used method consists of diluting 4 ml of *R.officinalis* oil in 40 ml of acetone. This solution was mixed with 40g of powder (containing 20g of gum Arabic and 20g of chitosan). After 5 min of manual stirring, the mixture was placed in a water bath at 30 °C until the complete evaporation of acetone (obtaining a dry flavored powder). Likewise, a suspension of the powder mixture (gum Arabic + chitosan) with 40ml of acetone without essential oil was also prepared to serve as a control.

The flavored powders were stored in brown bottles and tightly closed using parafilm and placed in the refrigerator at 4 ° C.

2.5. Characterization of the formulation

2.5.1. Encapsulation Efficiency (EE) and Loading Capacity (LC)

The percentages of EE and LC were determined according to the method described by Woranuch and Yoksan[12]with some modifications, 10 mg of flavored dry chitosan-gum Arabic was mixed with a solution of hydraulic acid (HCl) (2 M, 4 mL) and boiled at 95 °C for 30 min. Two ml of ethanol was added after cooling. Then, the mixture was centrifuged at 24,000 rpm for 30 minutes at 4 °C to separate the loaded pellet from the aqueous solution. One ml of supernatant was measured by spectrophotometer at 285 nm. A blank was prepared from chitosan-gum Arabic prepared without essential oil. The amount of essential oil charged was calculated from a calibration curve prepared with *R. officinalis* oil in 95% ethyl alcohol (Abs = 006 [conc] + 0.220; R^2 = 0.577). Each sample was measured three times.

2.5.2. Bioassays of the insecticidal activity of the Chitosan-EO and non-encapsulated EO

For free and encapsulated essential oil, the test consists of introducing 1 adult for each 10 g of substrate into glass bottle of 1000 mL volume containing 420 g of semolina. The oil was deposit using a micropipette on disks of filter paper 7.5 cm of diameter (Whatman N°1 paper) with the concentration 245.82 µL/ L air. For encapsulated essential oil the fumigation test was carried out, of which 3.4 g and 1.75 g of chitosan-EO capsule were usedrespectively against *T. castaneum.* The capsules were put in a thin tissue which is then glued to the wall of the jar which is closed hermetically. Each test has 3 replicates. Untreated tests serving as a control were maintained under the same conditions. Mortality assessment was carried out after 30, 45 and 60 days of exposure.

2.6. Statistical analyzes

Data were analyzed using SPSS software (version 20). The analysis of variance was carried out according to the GLM (General Linear Models) procedure and the comparison of the means was carried out by the Duncan test at the 5% probability threshold. Data were arranged according to the independent variables: treatment, and storageperiod. The dependent variable was the *T. castaneum*adult mortality rate.

3. Results

3.1. Encapsulation efficiency (EE) and loading capacity (LC)

Table 1presented the encapsulation efficiency and loading capacity of the two ratios chitosan: essential oil: 1: 0.5 and 1: 0.2.

Table 1. Encapsulation efficiency (EE) and loading capacity (LC) of *Rosmarinus officinalis* essential oil measured by spectrophotometer (UV).

Chitosan: EO (P/P)	EE (%)	CC(%)
Ratio .1.0:0.5.	35.8	2.49

EE = (mass of loaded oil / initial mass of oil) * 100

CC = (mass of charged oil / mass of sample) * 100

The encapsulation efficiency (EE) and loading capacity (CC) for the essential oil of *R. officinalis* encapsulated in a chitosan matrix have been presented in Table 1. In this work, the average value of the EE was 35.8%. Further, the results indicated that the CC value reached 2.49%.

3.2. Mortality evaluation of insects treated with chitosan-essential oil and crude essential oil

Figure 1 shows the mortality percentages of adults of *T.castaneum* after 30, 45 and 60 days of storage periods exposed to crude and encapsulated essential oil.

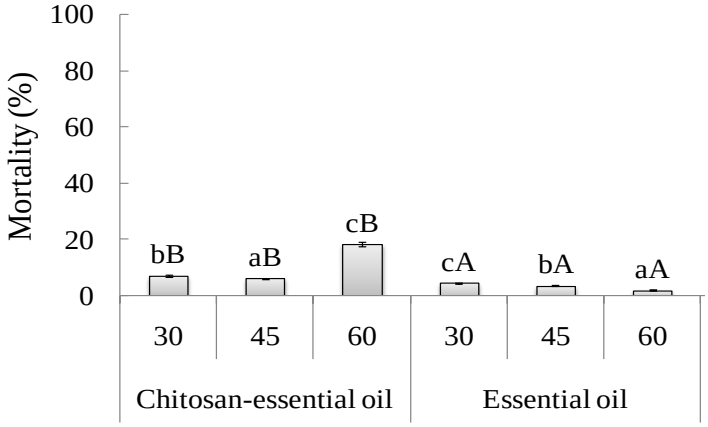

Figure 1. Mortality (%) of *Tribolium castaneum* adults caused by *Rosmarinus*officinalis *essential* oil, encapsulated chitosan-essential oil and chemical treatment

Results show that the mortality rate varies depending on the storage period. The mortality rate of *T. castaneum* during the three storage periods (30, 45 and 60 days) varies between 8% and 20% for encapsulated essential oil against 6.7and 2.1% for crude rosemary oil, which shows the effectiveness of the formulation over time.

From the results, we can notice a strong resistance of *T. castaneum*against essential oil compared to chitosan-essential oil.Statistical analyzes indicate significant differences between the different storage periods ($F = 19.5$; $df = 2$; $P = 0.000$). Similarly, the results reveal highly significant differences between the two treatments ($F = 4374.00$; $df = 1$; $P = 0.000$).

4. Discussion

Essential oils could be efficient alternatives to conventional fumigants because of their toxicity against insect pests of stored products [13]. However due to their fast degradability properties [14], other appropriate methods are well needed such as encapsulation. Based on these results, the formulation chitosan-essential oil showed encapsulation efficiency with 35.8% for the ratio 1.0:0.5. In accordance with this study [15] reported that encapsulation efficiency of chitosan-essential oil was 37.87%. However, the nanogels based on chitosan and cachew gum at the ratios matrix: oil 10:2, gum: chitosan 1:1 showed high encapsulation efficiency (70%). On the other hand, thiswork indicated that mortality of adults caused by chitosan-rosmery essential reached its maximum 20% after 60 days of storage. Nevertheless, the extension of storage period causes the decrease of the toxicity of essential oil. These results are in agreement with those of Abada et al. [16] that showed the negative correlation between toxicity of rosemary essential oil against*T. castaneum*and storage period.

5. Conclusion

It could be conclude that the insecticidal activity of encapsulated essential oil was more toxic against *T. castaneum* better than crude essential oil. Thus, formulation by using chitosan matrix may improve the toxicity of essential oil during storage. Additionally, this

technique is needed to overcome the constraints of essential oils in control of stored products. While, additional experiments are recommended to improve of the encapsulation efficiency and clarify its potential toxicity.

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