Assessing the Insecticidal Impact of Rosemary Essential Oils on the Saw-toothed Grain Beetle *Oryzeaphilus surinamensis*

Abir Soltani, Soumaya Haouel-Hamdi, **Insaf Ajmi**, **Maha Ben Abada, Tasnim Djebbi, Hadhami Chargui, Imen Mathlouthi, Amina Laabidi,** Laboratoire de Biotechnologie Appliquée à l'Agriculture (LR11INRAT06), Institut National de la Recherche Agronomique de Tunisie, Université de Carthage, Rue Hedi Karray, 1004 El Menzah, Tunisia, **Hela Mahmoudi,** Département de Biologie, Institut Supérieur de Biotechnologie de Béja, Université de Jendouba, Beja, Tunisia, **Jouda Mediouni-Ben Jemâa** Laboratoire de Biotechnologie Appliquée à l'Agriculture (LR11INRAT06), Institut National de la Recherche Agronomique de Tunisie, Université de Carthage, Rue Hedi Karray, 1004 El Menzah, Tunisia

https://doi.org/10.52543/tjpp.17.1.2 (*Tunisia*)

ABSTRACT

Soltani, A., Haouel-Hamdi, S., Ajmi, I., Ben Abada, M., Djebbi, T., Chargui, H., Mathlouthi, I., Laabidi, A., Mahmoudi, H., and Mediouni-Ben Jemâa, J. 2022. Assessing the insecticidal impact of rosemary essential oils on the saw-toothed grain beetle *Oryzeaphilus surinamensis*. Tunisian Journal of Plant Protection 17 (1): 15-28.

This work studied the fumigant toxicity of free and encapsulated rosemary (*Rosmarinus officinalis*) essential oils against adults of the saw-toothed grain beetle (*Oryzeaphilus surinamensis*) for three storage periods: 30, 45 and 60 days. Chitosan was used as encapsulation matrix. GC/MS analysis results showed that camphor and 1,8-cineole were the major components with respectively 18.04% and 39.67%. Mortality rates caused by the essential oils at 300 µL/L air after 10 days of storage were about 85.48%. The median lethal concentration (LC_{50}) was 124.80 µL/ L air. Encapsulation efficacy was 25.8% and loading capacity was 1.9%. Encapsulated essential oils achieved an efficacy of 82%, 100% and 100% respectively after 30, 45 and 60 days of storage. Reference treatment with Phosphine revealed a toxicity of 100%, 96% and 71% after 30, 45 and 60 days of storage respectively. Results showed that encapsulated essential oils caused a very slight modification on semolina properties. Protein contents decreased at the end of the storage period less than 1% (from 13.61% after 30 days to 12.91% after 60 days of storage). Encapsulated essential oils might be considered as an alternative fumigant control way for semolina without deterioration of its quality during storage.

Keywords: Chitosan, encapsulation, fumigant toxicity, *Oryzeaphilus surinamensis*, *Rosmarinus officinalis*, semolina quality

Durum wheat (*Triticum turgidum*) is the basic component of the daily human food worldwide (Durante et al. 2012; Shewry and Hey 2015). It provides carbohydrates, food calories and nutriments such as vitamin E as tocopherols and tocotrienols (Breiman and Graur 1995; De Santis et al. 2021). Even though, Bushuk (1997) and Pompa et al. (2021) reported that durum wheat constitutes only 5% to 8% of world production, it is considered as one of the

Corresponding author: Abir Soltani
Email: soltany.aby@gmail.com

Accepted for publication 26 April 2022

most economically important crop. The economic and dietary importance of this crop, forced the researchers as well as producers to focus on improving its production in the field and its conservation during storage (Saari and Prescott 1985). Durum wheat productivity is profoundly affected by biotic and abiotic stresses (Xynias et al. 2020). In this context, insects are considered as the most damaging pests of stored cereals (Alonso-Amelot and Avila-Núñez 2011). Ahmed (1983) and Hinton (1945) revealed that stored products may be attacked by more than 600 of beetles' species. Various treatments and technologies have been adopted in order to overcome the problems especially stored insect pests (Owusu 2000).

Rosemary (*Rosmarinus officinalis*) is a botanical species known by its a insecticidal potential against insects during storage, due to its major component such as 1,8-cineole, α-pinene, camphor, camphene (Katerinopoulos et al. 2005; Krzyżowski et al. 2020; Silvestre et al. 2019). Due to its properties such as volatility, absorption, bioavailability and tolerance to storage conditions such as temperature and oxygen, essential oils of rosemary are used as an alternative way of pest control (Turasan et al. 2015; Yadav et al. 2017)). Alternative methods are well needed such as encapsulation by using different matrix, which is considered as one of the most effective technique that preserve essential oils (Raza et al. 2020). Previous works demonstrated that chitosan matrix is a good approach to preserve essential oils properties and improve its insecticidal toxicity during storage (Salar et al. 2019).

The objective of this work was (i) to evaluate the fumigant toxicity of rosemary essential oils against one important stored durum wheat insect pest, the saw-toothed grain beetle, *Oryzeaphilus surrinamensis* (Silvanidae, Coleoptera), during different storage periods 30, 45 and 60 days, and (ii) to determine the insecticidal potential of encapsulated rosemary essential oils into chitosan matrix. Consequently, these objectives show the way of another objective, which aspire to understand the effect of those free and encapsulated essential oils of rosemary on some physical and chemical properties of the treated semolina (humidity, gluten, protein, and lipid) during the storage periods.

MATERIALS AND METHODS

Plant material and essential oil extraction.

Aerial parts of rosemary leaves were collected from the region of Korbos (846 m 36° 48'54" N 10° 34' 14" E) situated in North Tunisia during February and March 2021. Leaves were dried at room temperature for 10 days at darkness before distillation (Soltani et al. 2020). The extraction was made by hydrodistillation using Clevenger-type apparatus during 4 hours.

Gas Chromatography-Mass Spectrometry (GC-MS).

Quantitative and qualitative analysis of the essential oils was performed using an Agilent-technologies CPG-SM as described in previous research reported by Soltani et al. (2020).

Insect rearing.

Adults were collected from the rearing colony carried out in laboratory of the National Institute of Agronomic Research of Tunisia (INRAT). Rearing of *O. surinamensis* was carried out on semolina in a temperature of 25± 1°C, a relative humidity of 65 ± 5% and a photoperiod of 12 h Light/12 h Dark (Dal Bello et al. 2000; Soltani et al. 2020). The boxes were covered with muslin cloth. After 14 days, adults were removed by sieving.

Preliminary bioassay.

The preliminary fumigant test was conducted by using three doses 26.3, 52.5 and 105 µL; in order to evaluate the toxicity and to determine the median lethal concentration (LC_{50}) after 10 days of storage with occupation of 100%. A density of 1 adult newly emerged were placed per 10 g into 1000 mL glass bottles contained 420 g of semolina during 10 days of storage. Essential oils were deposited on filter paper disks of 7.5 cm² (Whatman N° 1) using a micropipette. These doses were converted into concentrations, namely 75, 150 and 300 µL/L air. Corrected mortality percentages were calculated by using Abbott's (1925) formula. Additionally, the lethal concentration CL_{50} and CL_{95} were evaluated using the Probit Analysis (Finney 1971).

Encapsulation.

The formulation was performed according to the protocol of Keita (2010) with some modifications. Rosemary essential oils were diluted in acetone at concentration (10%). The obtained solution was mixed with gum Arabic and chitosan. Two ratios of chitosan: gum Arabic: essential oils (w/w/w) were used namely 0.5:0.5:0.5 and 0.5:0.5:0.25. After 5 min of manual stirring, the mixture was placed in water bath at 30 °C until complete evaporation of acetone. Similarly, a suspension of the powder (gum Arabic and chitosan) was mixed only with acetone in order to serve as a control. The flavored powders were stored in brown bottles and tightly closed using parafilm and placed at 4 °C.

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

Encapsulation Efficiency (EE %) and Loading Capacity (LC %) were calculated according to the methods and formula described by Keawchaoon and Yoksan (2011) with some modifications. The amount of essential oils charged was determined from a calibration curve prepared with rosemary essential oils in 95% ethyl alcohol (Abs = 006 [conc] + 0.220; R^2 = 0.577). Each sample was measured three times. These parameters were calculated according to the following formula:

$$EE\ (\%) = \frac{mass\ of\ loaded\ oils}{initial\ mass\ of\ oils} \times 100$$

and

$$LC\ (\%) = \frac{mass\ of\ charged\ oils}{mass\ of\ sample} \times 100$$

FTIR characterization.

Fourier-Transform InfraRed (FTIR) spectroscopy was used to determine the information about functional groups or chemical band in the samples. The FTIR spectra of the microcapsules were achieved by using a Fourier-Transform infrared spectro-photometer JASCO (FT/IR4700 type A). Dried samples of chitosan: gum Arabic and chitosan: gum Arabic: essential oils at two ratio (1:1:0.2; 1:1:0.5) were analyzed. The analysis was accomplished as cited by Chaib et al. (2021). Each spectrum was recorded in a frequency range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}.

Fumigation tests for free and encapsulated essential oils.

A number of 42 newly emerged adults of *O. surinamensis* were placed in 1000 mL glass bottles containing 420 g of semolina. Regarding the free essential oils, the bottle was secured as described in previous section, the used concentration was the same used in the preliminary bioassay. The insecticidal activity of the formulation chitosan: gum Arabic: essential oils was determined according to the protocol described by Soltani et al. (2022). A mass of 1.75 g of capsule was

placed in a thin tissue that was glued to the suburb of the bottle and closed hermetically. Tests were replicated three times for each treatment. Untreated boxes were used as a control. Treated and untreated boxes were placed under the same conditions. Mortality assessment was carried out after 30, 45 and 60 days of storage. Phosphine (PHOSTOXIN®) was a chemical treatment that used as a reference at doses of 3 mg/L air.

The inhibition emergence percentage was determined using the method from Tapondjou et al. (2003) described by the following formula:

Inhibition (%) = $(Cn - Tn)/Cn*100$

where Cn = number of adults emerged in untreated boxes (control) and Tn = number of adults emerged in the boxes treated with the essential oils.

Proximate composition.

The impact of free and encapsulated essential oils as well as chemical treatment (Phosphine) on semolina properties such as humidity, gluten, protein, and lipid were evaluated according to the standard methods of analysis (AOAC 1984).

Protein content.

Protein content was quantified through the determination of the total nitrogen determined according to Kjeldahl method (1883). This method consists of three phases: mineralization, distillation and titration. Around 1 g of powder dates was hydrolyzed with 15 mL concentrated sulfuric acid (H_2SO_4) containing two copper catalyst tablets in a heat block at 420 °C for 2 h. Behind cooling, we added H_2O to the hydrolysate thereafter, neutralization and titration were done. The total nitrogen was calculated according to the formula:

$$Total\ nitrogen\ (\%) = \frac{VHCl * 0.0875}{WRow\ material}$$

where V_{HCL} = Volume of HCl and $W_{Row\ material}$: Weight of row material.

The protein content was calculated by multiplying the total nitrogen rate N (%) by the coefficient 6.25 (Thabet et al. 2007):

Protein content = Total nitrogen content × 6.25.

Lipid content.

Extraction of free lipids from durum wheat semolina was carried out according to the method described by Ounane et al. (2006) with some modifications. The extraction was performed into Soxhlet extraction apparatus by using ether. In fact, 20 g of substrate were introduced into cellulose cartridge. A volume of 60 ml of ether was placed into bucket that was stirred at 110°C until exhaustion of the lipids entrained by the organic solvent. The semolina was dried in a vacuum oven at 60 °C for 24 h to remove traces of solvent. Then, the buckets were weighed again. The lipid content was determined by the difference in mass. All measurements were repeated thrice.

Statistical analyses.

Data were analyzed using SPSS statistical software version "20". For each parameter, mortality percentage and semolina property data were analyzed using two-way ANOVA.

RESULTS

The chromatographic analyses (GC-MS) of rosemary essential oils indicated the presence of 23 components with a percentage of about 99.3%. The identified components were divided into three chemical classes (Table 1). The major component (> 5 %) were identified by matching their spectra with accessible ones according to the database of bibliography. The major class was represented by oxygenated monoterpenes

(58.87%), followed by sesquiterpenes hydrocarbons (21.36 %) and oxygenated monoterpenes (12.27 %). Rosemary leaves were characterized by the highest fraction of 1,8-cineole with (39.67 %), followed by camphor (18.04 %) and bornneol (10.5 %). The obtained retention indexes (RI^a /RI^b) are summarized in Table 1.

Table 1. Chemical compounds and identified constituents (%) of essential oils extracted from rosemary

N°	Component	Fraction (%)	RI^a /RI^b
	Monoterpene hydrocarbons	**12.27**	
1	α-Pinene	6.33	937/913
2	α-Terpinene	0.39	1021/-
3	β-pinene	2.84	982/965
4	γ-Terpinene	0.7	1107/1041
5	ʋ-Terpinolene	0.62	1092/-
6	β-Myrcene	0.93	992/991
7	Bornylacetane	2.52	
	Oxygenated Monoterpenes	**58.87**	
8	O-cymene	1.33	1028/-
9	1,8-Cineole	39.67	**1036**/1041
10	Borneol	10.51	1185/1164
11	Carvacrol	0.86	1433/-
12	Linalool	1.48	1151/1175
13	Terpinene-4-ol	1.31	1200/1164
14	ʋ-terpineol	4.17	1208/-
	Sesquiterpenes hydrocarbons	**21.36**	
15	α-Caryophyllene	0.22	1459/-
16	β-Caryophyllene	3.1	1429/1421
17	Camphor	18.04	1175/1119
	Other compounds	**6.88**	
18	Heptane	2.58	
19	Cycloheptasiloxane	0.33	
20	1,2-Phenylene	1.02	
21	Tétracyclohexane	0.25	
22	α-Phellandrène	0.18	
	Minor compounds	**1.83**	
	Major compounds	**97.5**	
	Total identified compounds (%)	**99.33**	

RI^a: Retention Index determined according to the homologous series of n-alkanes (C9-C24); RI^b; Literature Retention Index.

Preliminary Bioassay.

Exposed *O. surrinamensis* adults to free essential oils during 10 days of storage showed different mortality rates at different concentrations of free essential oils (Fig 1). Results showed that mortality rate increased with increasing concentration with significant differences between concentrations ($df = 2$, $f = 896.12$, $p \leq 0.01$).

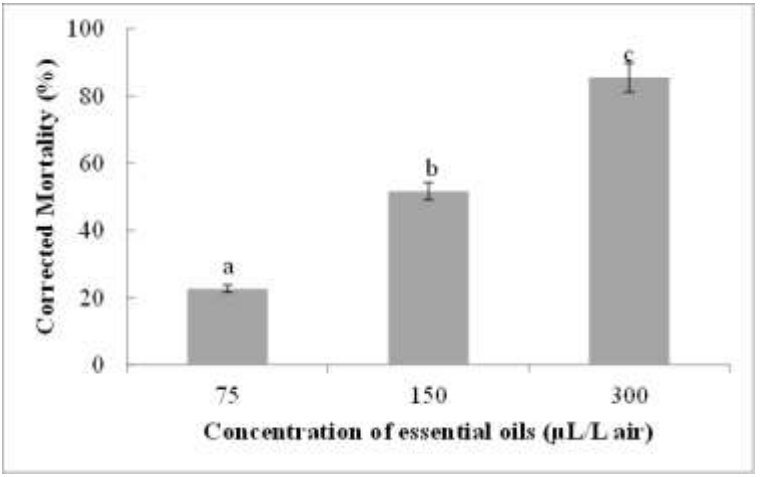

Fig. 1. Corrected mortality rates of *Oryzeaphilus surinamensis* during 10 days of storage treated by rosemary essential oils. Comparison was made between concentrations. The values followed by different letters are significantly different ($p \leq 0.05$).

Regarding lethal concentrations (LC_{50} and LC_{95}) of rosemary essential oils after 10 days of storage, obtained results are shown in Table 2. The LC_{50} and LC_{95} values of the essential oils were respectively 124.8 and 231.3 µL/L air. The median lethal concentration was used for the fumigation and encapsulation.

Table 2. LC_{50} and LC_{95} values (µL/L air) of rosemary essential oils from the North West of Tunisia after 10 days of storage used against *Oryzeaphilus surrinamensis*.

Insect	Slope ± ES*	χ^2	LC_{50}**(µL/L air)	LC_{95}***(µL/L air)
Oryzaephilus surinamensis	0.015±0.002	5.85	124.80	231.30

Data tested by χ^2-test for homogeneity of 1:1 ratio; * ES = Standard error, **LC_{50} = Median lethal concentration; *** LC_{95} =Lethal concentration at 95% of the insect population.

Encapsulation efficiency (EE %) and loading capacity (LC %) for two ratio chitosan: gum Arabic: Essential oils with (w:w:w; 0.5:0.5:0.5 and 0.5:0.5:0.25) are shown in Table 3. Highest values of EE% and LC% were about 25.8 and 1.49% recorded for Ratio 1 (0.5:0.5:0.5).

Table 3. Encapsulation efficiency (EE %) and Loaded capacity (LC %) of rosemary essential oils (EO) loaded in chitosan matrix determined by UV-Vis spectrophotometry

Chitosan : EO (W/W)	EE (%)	LC (%)
R1=0.5:0.5:0.5	25.8	1.49
R2=0.5:0.5:0.25	12.9	0.6

Fourier Tranfsorm Infrared Spectroscopy (FTIR) characterization.

Fig 2 reported the FTIR spectra of chitosan: gum Arabic (50%) and rosemary essential oil loaded into chitosan and gum Arabic matrix with ratio 0.5:0.5:0.5 and 0.5:0.5:0.25.

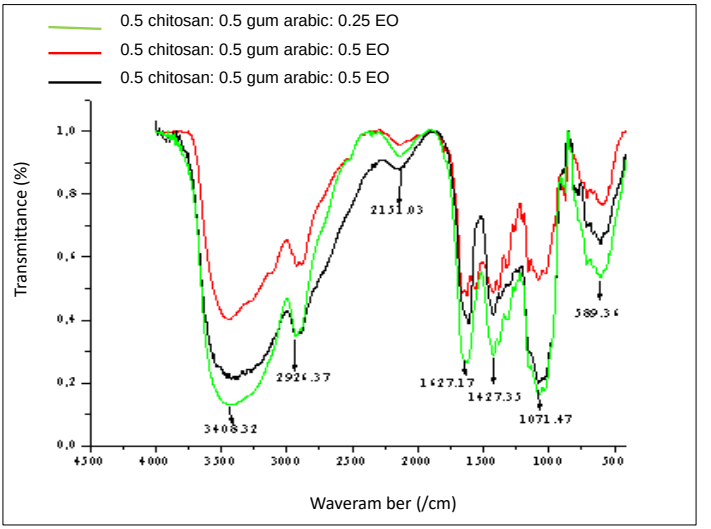

Fig. 2. Spectro FTIR of chitosan: gum Arabic for one ratio 0.5: 0.5 and chitosan: gum Arabic: Essential oils for two ratios 1 :0.5 et 1 :0.2.

In general, chitosan powder revealed peaks characteristics. Results showed peaks at 35779 (-OH and NH_2), at 2947(-CH), 1631 (Amide I), 1438 (C-O-C) and finally a characteristic peak by the establishment of a complex through an interaction between NH_3^+ groups and chitosan: gum Arabic powder at 389.76 and 3474 cm^{-1} respectively for the two ratios 0.5:0.5:0.5 and 0.5:0.5:0.25. Besides, new peaks appeared at 1056.59, 1353.98, 1025.3 and 935.1 cm^{-1}. In fact, obtained results confirm those reported by Hosseini et al. (2013) and Yoksan et al. (2010). These authors revealed that appearance of new peaks of C-O-C and the appearance of Amide II are due to the interaction between the NH_3^+ groups of chitosan and the chemical group within the formulation.

Fumigant toxicity and comparison between free and encapsulated essential oil effect against *O. surinamensis.*

Corrected mortality rates obtained for *O. surinamensis* showed different results according to adults during three storage periods (30, 45 and 60 days under chemical), free essential oils, chitosan: essential oils treatments.

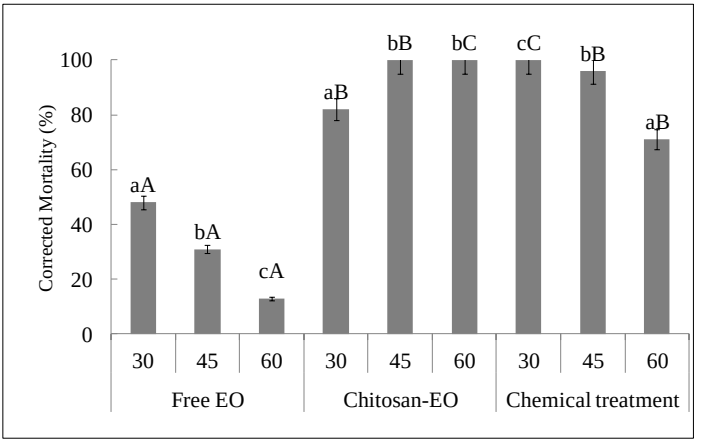

Fig. 3. Mortality percentages of *Oryzeaphilus surrinamensis* caused by free and encapsulated essential oils during storage periods of 30, 45 and 60 days. Bars represent means ± standard error of three replicates. Different letters a, b and c indicated significant differences at ($p < 0.001$) for storage periods; A, B and C indicated significant differences at ($p < 0.01$) for treatment according Duncan test).

O. surinamensis varied in their susceptibility to treatments and storage periods. Mortality percentage for the control was 0%, 2.31% and 4.17% after 30, 45 and 60 days of storage. Corrected mortality was high for chitosan: essential oils and chemical treatments under the same conditions. However, for chemical treatment, there was a decrease of mortality percentages from 100% after 30 days to 61.2% after 60 days of storage. Likewise, for free essential oils the mortality percentages were 48% and 13% after 30 and 60 days of storage. Statistical analyses revealed high significant differences between treatments against *O. surinamensis* during different storage periods (df = 2, f = 28357.28, $p \leq 0.001$). Equally, high significant differences on mortality percentages were observed between storage periods for free essential oil treatment (df = 3, f = 1654.06, $p \leq 0.001$). For encapsulated essential oils and chemical treatment, differences were observed (at $p \leq 0.05$). At the same time, the results obtained for inhibition of emergence was zero after 30, 45 and 60 days of exposure for encapsulated essential oils. On the other hand, emergence inhibition rate was 0% after 30 days and reached 5.36% and 8.12% after 45 and 60 days of storage for chemical treatment, respectively. However, free oils present an inhibition rate of 21.54%, 36.1% and 43.12% after 30, 45 and 60 days of storage, respectively.

Impact of infestation by *O. surinamensis* and different treatments on semolina quality.

Impact of free and encapsulated essential oils as well as phosphine treatment on humidity, Gluten, Protein and Lipid are reported in Table 4.

Table 4. Properties of semolina treated with free and encapsulated essential oils (EO) compared to phosphine.

Treatment	Storage periods	Humidity (%)	Gluten (%)	Protein (%)	Lipid (%)
Free EO	30	11.7±0.57 a	25.2±1.0 c	13.49±0.01 c	1.75±0.5 b
	45	12.1±0.57 b	24.2±1.51 b	13.0±0.34 b	0.92±0.35 a
	60	12.8±0.57 b	22.7±1.06 a	12.7±0.71a	0.82±0.52 a
Encapsulated EO	30	10.02±1.15 a	26.2±1.53 c	13.61±0.5 b	1.81±0.57 b
	45	11.98±1.15 b	24.1±1.75 b	13.26±0.5 b	0.98±0.57 a
	60	12.03±1.15 c	23.0±1.52 a	12.91±0.57 a	0.91±0.6 a
Chemical treatment (Phosphine)	30	10.91±0.57 a	25.1±1.0 c	13.14±0.01 b	1.71±0.5 c
	45	11.2±0.57 b	24.1±1.0 b	12.89±0.1 a	1.01±0.5b
	60	12.5±1.15 c	22.9±1.2 a	12.64±0.01 a	0.89±0.5 a
Infested and not treated	30	13.5±0 a	23.8±1.53 c	11.1±0.01 b	1.15±0.01 b
	45	13.7±0.57 b	22.4±1.2 b	11.03±0.01 b	0.72±0.01 a
	60	14.02±0 c	21.1±1.50 a	10.8±0.01 a	0.7±0.01 a
Control	30	12.01±0.6 b	26.8±2.1 b	13.4±0.01a	1.9±0.57 a
	45	11.08±0.57 a	23.1±2.18 a	13.3±0.01 a	1.7±0.6 a
	60	11.03±0.57 a	23.7±1.9 a	13.1±0.57 a	1.05±0.5a7

For each treatment, values followed by different letters are significantly different at $p < 0.5$.

Chemical compositions of untreated and treated semolina with free and encapsulated essential oils, and chemical treatments during different storage periods are reported in Table 4. Results revealed that storage periods and treatments have an effect on the semolina quality.

The humidity percentage of semolina increased during storage periods. In fact, it was about 11.7 % for semolina treated with essential oils, 10.02 % for semolina treated with chitosan: essential oils, 10.91 % for semolina treated by phosphine, 13.7 % for infested semolina and 12.01% for the control, after 30 days. And the increase reaches 12.8, 12.03, 12.5, 14.02, 11.03 % after 60 storage days for semolina treated with essential oils, chitosan: essential oils, phosphine, infested semolina and the control, respectively. Besides, significant differences at $p \leq 0.05$ in humidity were recorded between treatments.

The gluten content of semolina decreased during storage from 25.2, 26.2, 23.8 and 26.8 after 30 days to 22.7, 23.0, 22.9, 21.1 and 23.7 after 60 days of storage for free essential oils, chitosan: essential oils, phosphine, infested semolina and control, respectively. Significant differences have also been observed between treatments (df = 3, f = 2.92, $p \leq 0.03$). In addition, the highest gluten amount was observed for semolina treated with encapsulated essential oils after 30 days of storage (Table 4), while lowest value was recorded for infested semolina after 60 days of storage. Thus, gluten content showed no significant differences ($p \geq 0.05$) during the storage.

The protein contents of semolina stored during different periods under various treatments were reported in Table 4. The protein content in infested semolina was 11.1, 11.03 and 10.8% after 30, 45 and 60 days of storage, respectively. However, for treated semolina with chitosan: gum Arabic: essential oil protein content reached its maximum 13.61% after 30 days of storage. A decrease on protein values have been observed after 45 and 60 days of storage (Table 4). The storage period tended to cause a decrease in protein content of semolina. Significant

differences were registered between treatments (df = 4, f = 66.87, $p \leq 0.01$).

The lipid percentages of semolina during storage periods ranged between 0.7 and 1.15 for infested semolina while it was comprised between 1.05 and 1.9 for control. Furthermore, for treated semolina with free essential oils, chitosan: gum Arabic: essential oils and chemical treatment lipid content varied from 0.82 to 1.75 and from 0.91 to 1.81 and from 0.89 to 1.71, respectively. No significant differences were observed on lipid content between treatments (df = 4, f = 2.04, $p \leq 0.1$). Moreover, significant differences were registered between storage periods (df = 2, f = 0.3, $p \leq 0.01$).

DISCUSSION

During this study, the GC-MS analysis was conducted to determine chemical composition of rosemary essential oils. Results showed that 1,8-Cineole, Borneol, and α–Pineneas are the major components with more than 5% of rosemary essential oils, and which can be confirmed by previous work conducted on rosemary by Kadri et al. (2011). According to Prakash et al. (2021), different factors may affect the percentages of the chemical components such as plant species, parts of plant, seasons, extraction methods and geographical conditions. Subsequently, the median lethal concentration will be used for encapsulation with chitosan and gum Arabic matrix. In fact, the encapsulation inhibits the degradation of food ingredients under different conditions such as oxygen, heat and moisture (Maswal and Dar 2014). According to Taylor et al. (2007), the increase of matrix concentration makes encapsulation efficiency values significantly increase. One of the most known matrix chitosan-encapsulated essential oils could be utilized as an encapsulating matrix due to various characteristics such as ecological safety, low toxicity and excellent biodegradability (Prashanth and Tharanathan 2007; Zhang et al. 2022). This study has been made in order to evaluate the efficacy of free and encapsulated rosemary essential oils as an ecofriendly alternative to chemical treatment like phosphine. Due to their major component like 1,8-cineole, these oils had a high fumigant toxicity for controlling *O. surinamensis*. In the same context, Lee et al. (2002) demonstrated that rosemary essential oils showed high fumigant toxicity against insect pests of stored products, whereas results revealed that toxicity potential of free essential oils depend on storage durations. An interesting decrease has been shown on corrected mortality caused by free essential oils from 4 % after 30 days to 3 % after 45 days to reach finally 1 % after 60 days of storage. This is due probably to the volatile compounds. During three storage periods, it has been shown that encapsulated essential oils were relatively more toxic against *O. surinamensis*. In addition, considerable differences were observed in mortality of insects between different treatments. Regarding fumigant test, toxicity of encapsulated essential oils is due to major components. According to previous researches, major compounds such as phenol, camphor and 1,8-cineole had a high insecticidal toxicity against various insect pests (López and Pascual-Villalobos 2010).

The humidity percentage of semolina in the present study varied upon storage durations. These results showed that chemical proprieties of semolina changed during storage periods with deterioration on semolina quality in infested semolina by *O. surinamensis* compared to control and treated semolina. Results showed that lowest values were registered in infested semolina with

chemical factors such as gluten, protein, and lipid. Similarly, Haouel-Hamdi et al. (2020), Saeed Mohamed et al. (2012), Panth and Susheela (1977) revealed that infestation by insects during storage periods cause high deterioration and damages in cereal. In addition, Sanchez-Marinez et al. (1997) reported that insect infestation leads to protein degradation and modification in gluten structure. Results showed high variation in gluten quantity in infested semolina compared to control and treated semolina. Furthermore, these results are confirmed by those reported by Mohammad et al. (2012), which demonstrated that insect infestation caused the decline in the gluten content which gives the flour certain liquidity and lack of rubber and cohesion. The results regarding protein contents during different storage periods were less than those obtained in earlier study conducted by Erbas et al. (2005), which revealed that protein percentage (15.35%) decreased through the storage periods, but our results confirm those reported by Haouel-Hamdi et al. (2020).

RESUME

Soltani A., Haouel-Hamdi S., Ajmi I., Ben Abada M., Djebbi T., Chargui H., Mathlouthi I., Laabidi A., Mahmoudi H. et Mediouni-Ben Jemâa J. 2022. Évaluation de l'impact insecticide des huiles essentielles du romarin sur le cucujide dents de scie des grains *Oryzeaphilus surinamensis*. Tunisian Journal of Plant Protection 17 (1): 15-28.

L'étude explore la toxicité fumigène des huiles essentielles du romarin (*Rosmarinus officinalis*) libres et encapsulées contre les adultes du cucujide dents de scie des grains (*Oryzeaphilus surinamensis*) pendant trois périodes de stockage, 30, 45 et 60 jours. Le chitosane a été utilisé comme matrice d'encapsulation. Les résultats GC/MS ont montré que le 1,8-cinéole et le camphre étaient les principaux composants avec 39.67% et 18.04%, respectivement. Les huiles essentielles ont causé une mortalité de 85.48% à 300 µL/L air après 10 jours de stockage. La concentration létale médiane (CL_{50}) était de 124,80 µL/L d'air. L'efficacité d'encapsulation était de 25.8% et la capacité de charge était de 1.9%. Les huiles essentielles encapsulées ont atteint une efficacité de 82%, 100% et 100% après 30, 45 et 60 jours de stockage, respectivement. Le traitement de référence à la phosphine a révélé une toxicité de 100%, 96% et 71% après 30, 45 et 60 jours de stockage. En revanche, les résultats ont montré que les huiles essentielles encapsulées entraînaient une très faible modification sur les proprietes de la semoule. Les teneurs en protéine ont diminué à la fin de la durée de stockage de moins de 1% seulement (de 13.61% après 30 jours à 12.91% après 60 jours de stockage). Ainsi, les huiles essentielles encapsulées pourraient être considérées comme un moyen de fumigation alternatif pour la semoule sans détérioration de sa qualité pendant le stockage.

Mots clés: Chitosane, encapsulationqualité de la semoule, *Oryzeaphilus surinamensis*, *Rosmarinus officinalis*, toxicité de fumigants

ملخص

سلطاني، عبير وسمية حوال-حمدي وإنصاف عجمي ومهى بن عبادة وتسنيم دجبي وحذامي شرقي وإيمان مثلوثي وأمينة عبيدي وهالة محمودي وجودة مديوني بن جماعة. 2022. تقييم تأثير الزيوت العطرية لإكليل الجبل كمبيد حشري على خنفساء الحبوب المنشارية. Tunisian Journal of Plant Protection 17 (1): 15-28.

تستكشف الدراسة سمية التدخين للزيوت العطرية لإكليل الجبل (*Rosmarinus officinalis*) الحرة والمغلفة ضد الطور البالغ لخنفساء الحبوب المنشارية (*Oryzeaphilus surinamensis*) لمدة ثلاث فترات تخزين هي 30 و 45 و 60 يومًا. تم استخدام الشيتوزان كمصفوفة تغليف. أظهرت نتائج التحاليل الكيميائية GC/MS أن 1.8-سينيول والكافور كانا المكونان الرئيسيين بنسبة 39.67% و 18.04%، على التوالي. أظهرت الزيوت العطرية نسبة وفيات 85.48% عند تركيز 300

ميكرولتر/لتر هواء بعد 10 أيام من التخزين. كان متوسط التركيز المميت 124.80 ميكرولتر/لتر هواء. كانت فعالية التغليف 25.8% وسعة التحميل 1.9%. حققت الزيوت العطرية المغلفة 82% و 100% و 100% بعد 30 و 45 و 60 يومًا من التخزين، على التوالي. أظهرت المعاملة المرجعية بالفوسفين سمية 100% و 96% و 71% بعد 30 و 45 و 60 يوماً من التخزين. من ناحية أخرى، أوضحت النتائج أن الزيوت العطرية المغلفة تسبب تغيير طفيف في خصائص السميد، حيث انخفض محتوى البروتين في نهاية فترة التخزين بأقل من 1% فقط (من 13.61% بعد 30 يومًا إلى 12.61% بعد 60 يومًا من التخزين). وبالتالي، يمكن اعتبار الزيوت العطرية المغلفة وسيلة تبخير بديلة للسميد دون تدهور جودته أثناء التخزين.

كلمات مفتاحية: تغليف، جودة السميد، سمية التبخير، كيتوزان، *Oryzeaphilus surinamensis*، *Rosmarinus officinalis*

LITERATURE CITED

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. Journal of economical Entomology 18: 265-267

Ahmed, H. 1983. Losses incurred in stored food grains by insect pests-a review. Pakistan Journal of Agricultural Research 4: 198-207.

Alonso-Amelot, M.E., and Avila-Núñez, J.L. 2011. Comparison of seven methods for stored cereal losses to insects for their application in rural conditions. Journal of Stored Products Research 47: 82-87.

Breiman, A., and Graur, D. 1995. Wheat evolution. Israel Journal of Plant Sciences 43: 85-98.

Bushuk, W. 1997. Wheat breeding for end-product use. Pages 203-211. In: Wheat: Prospects for global improvement. Dordrecht, Netherlands.

Chaib, S., Benali, N., Arhab, R., Sadraoui Ajmi, I., Bendaoued, H., and Romdhane, M. 2021. Preparation of *Thymus vulgaris* Essential Oil Microcapsules by Complex Coacervation and Direct Emulsion: Synthesis, Characterization and Controlled Release Properties. Arabian Journal for Science and Engineering 46: 5429–5446.

Dal Bello, G., Padin, S., Lastra, C.L., and Fabrizio, M. 2000. Laboratory evaluation of chemical-biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains. Journal of Stored Products Research 37: 77-84

De Santis, M.A., Soccio, M., Laus, M.N., and Flagella, Z. 2021. Influence of Drought and Salt Stress on Durum Wheat Grain Quality and Composition: A Review. Plants 10: 2599.

Durante, M., Lenucci, M.S., Rescio, L., Mita, G., and Caretto, S. 2012. *Durum wheat* by-products as natural sources of valuable nutrients. Phytochemistry Reviews 11: 255-262.

Erbaş, M., Ertugay, M.F., and Certel, M. 2005. Moisture adsorption behaviour of semolina and farina. Journal of Food Engineering 69: 191-198.

Finney, D. 1971: Probit analysis 3rd ed Cambridge Univ. Press. London, UK. 32 pp.

Haouel-Hamdi, S., Hamedou, M.B., Bachrouch, O., Boushih, E., Zarroug, Y., Sriti, J., Messaoud, C., Hammami, M., Abderraba, M., Limam, F., and Mediouni Ben Jemâa, B. 2020. Susceptibility of *Tribolium castaneum* to *Laurus nobilis* essential oil and assessment on semolina quality. International Journal of Tropical Insect Science 40: 667-675.

Hinton, H. 1945. A Monograph of the beetles associated with stored products. Volume 1. A monograph of the beetles associated with stored products. British Museum, London, UK, 100 pp.

Hosseini, S.F., Zandi, M., Rezaei, and M., Farahmandghavi, F. 2013. Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: preparation, characterization and in vitro release study. Carbohydrate Polymers 95: 50-56.

Kadri, A., Zarai, Z., Chobba, I.B., Békir, A., Gharsallah, N., Damak, M., and Gdoura, R. 2011. Chemical constituents and antioxidant properties of *Rosmarinus officinalis* L. essential oil cultivated from the South-Western of Tunisia. Journal of Medicinal Plants Research *5*: 6502-6508.

Katerinopoulos, H.E., Pagona, G., Afratis, A., Stratigakis, N., and Roditakis, N. 2005. Composition and insect attracting activity of the essential oil of *Rosmarinus officinalis*. Journal of Chemical Ecology 31: 111-122.

Keawchaoon, L., Yoksan, R. 2011. Preparation, characterization and in vitro release study of carvacrol-loaded chitosan nanoparticles. Colloids and Surfaces B: Biointerfaces 84:163-171

Kjeldahl, C. 1883. A new method for the determination of nitrogen in organic matter. Journal of Analytical Chemistry 22: 366.

Krzyżowski, M., Baran, B., Łozowski, B., and Francikowski, J. 2020. The effect of *Rosmarinus officinalis* essential oil fumigation on biochemical, behavioral, and physiological parameters of *Callosobruchus maculatus*. Insects 11: 344.

Lee, B. H., Lee, S.E., Annis, P.C., Pratt, S.J., Park, B.S., and Tumaalii, F. 2002. Fumigant toxicity

of essential oils and monoterpenes against the red flour beetle, *Tribolium castaneum* Herbst. Journal of Asia-Pacific Entomology 5: 237-240.

López, M., and Pascual-Villalobos, M. 2010. Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. Industrial Crops and Products 31: 284-288.

Maswal, M., and Dar, A.A. 2014. Formulation challenges in encapsulation and delivery of citral for improved food quality. Food Hydrocolloids 37: 182-195.

Mohammad, O.S., Ali, W.K., and Al-Hulitan, A.M.T. 2012. The effect of infestation by the confused flour beetle (*Tribolium confusum* Duv.) on specifications of wheat flour. Journal of Agricultural Science and Technology 2: 696.

Ounane, G., Cuq, B., Abecassis, J., Yesli, A., and Ounane, S.M. 2006. Effects of physicochemical characteristics and lipid distribution in Algerian *Durum wheat* semolinas on the technological quality of couscous. Cereal chemistry 83: 377-384.

Owusu, E.O. 2000. Effect of some Ghanaian plant components on control of two stored-product insect pests of cereals. Journal of Stored Products Research 37: 85-91.

Ferreira, T.P., Haddi, K., Corrêa, T.F.R., Zapata, V.L., Piau, T.B., Souza, L.F., Santos S.M.G., Oliveira, E.E., Jumbo, L.O.V., Ribeiro, B.M., Grisolia, C.K., Fidelis, R.R., Maia, A.M.S., and S. Aguiar, R.W. 2019. Prolonged mosquitocidal activity of *Siparuna guianensis* essential oil encapsulated in chitosan nanoparticles. PLoS Neglected Tropical Diseases 13: 1-23.

Finney, D. 1971. Statistical logic in the monitoring of reactions to therapeutic drugs. Methods of Information in Medicine 10: 237-245.

Pant, K.C., and Susheela, T.P. 1977. Effect of storage and insect infestation on the chemical composition and nutritive value of grain sorghums. Journal of the Science of Food and Agriculture 28: 963-970.

Pompa, C., D'Amore, T., Miedico, O., Preite, C., and Chiaravalle, A.E. 2021. Evaluation and Dietary Exposure Assessment of Selected Toxic Trace Elements in *Durum wheat* (Triticum durum) Imported into the Italian Market: Six Years of Official Controls. Foods 10: 775.

Prakash, V., Kumari, A., Kaur, H., Kumar, M., Gupta, S., and Bala, R. 2021. Chemical Composition and Anti-microbial Activity of Essential Oil from Aerial parts of *Origanum vulgare* L. from North-Western Himalayas. Journal of Essential Oil Bearing Plants 24: 177-185.

Prashanth, K.H., and Tharanathan, R. 2007. Chitin/chitosan: modifications and their unlimited application potential - An overview. Trends in Food Science and Technology 18: 117-131.

Raza, Z.A., Khalil, S., Ayub, A., and Banat, I.M. 2020. Recent developments in chitosan encapsulation of various active ingredients for multifunctional applications. Carbohydrate Research 492: 1-15.

Saari, E.E., and Prescott, J. 1985. World distribution in relation to economic losses. Page 259-298. In: Diseases, Distribution, Epidemiology, and Control. A.P. Roelfs, and W.R. Bushnell, Ed. Centro International de Mejoramiento de Matz y Trigo (CIMMYT), Mexico.

Salar, S., Jafari, M., Kaboli, S.F., and Mehrnejad, F. 2019. The role of intermolecular interactions on the encapsulation of human insulin into the chitosan and cholesterol-grafted chitosan polymers. Carbohydrate Polymers, 208: 345-355.

Sánchez-Mariñez, R., Cortez-Rocha, M., Ortega-Dorame, F., Morales-Valdes, M., and Silveira, M. 1997. End-use quality of flour from *Rhyzopertha dominica* infested wheat. Cereal Chemistry 74: 481-483.

Shewry, P.R., and Hey, S.J. 2015. The contribution of wheat to human diet and health. Food and Energy Security 4: 178-202.

Silvestre, W.P., Medeiros, F.R., Agostini, F., Toss, D., and Pauletti, G.F. 2019. Fractionation of rosemary (*Rosmarinus officinalis* L.) essential oil using vacuum fractional distillation. Journal of Food Science and Technology 56: 5422-5434.

Soltani, A., Aouadib, G., Yangui, I., Haddadi, S., Ben Gueayed, M., Boushih, E., and Mediouni Ben Jemâa, J. 2020. Fumigant toxicity of two Cineole-rich essential oils and their combinations for the control of *Carpophilus hemipterus* Linnaeus and *Sitophilus oryzae* Linnaeus. Annales de l'INRGREF 21 : 85-101

Soltani, A., Haouel-Hamdi, S., Sadraoui Ajmi, I., Djebbi, T., Ben Abada, M., Yangui, I., Chouachi, N., Hassine, K., Majdoub, H., Messaoud, C., and Mediouni Ben Jemâa, J. 2022. Insights for the control of dried-fruit beetle *Carpophilus hemipterus* (Nitidulidae) using rosemary essential oil loaded in chitosan nanoparticles. International Journal of Environmental Health Research 1: 1-11.

Taylor, T.M., Gaysinsky, S., Davidson, P.M., Bruce, B. D., and Weiss, J. 2007. Characterization of antimicrobial-bearing liposomes by ζ-potential, vesicle size, and encapsulation efficiency. Food Biophysics 2: 1-9.

Tapondjou, L., Lontsi, D., Luc, S.B., Jongwon, C., Kyung-Tae, L., Hyun-Ju, J., and Hee-Juhn, P. 2003. In Vivo anti-nociceptive and anti-inflammatory effect of the two triterpenes,

ursolic acid and 23-hydroxyursolic acid, fromcussonia bancoensis. Archives of Pharmacal Research 26: 143-146.

Turasan, H., Sahin, S., and Sumnu, G. 2015. Encapsulation of rosemary essential oil. LWT-Food Science and Technology 64: 112-119.

Yadav, E., Kumar, S., Mahant, S., Khatkar, S., and Rao, R. 2017. Tea tree oil: a promising essential oil. Journal of Essential Oil Research 29: 201-213.

Yoksan, R., Jirawutthiwongchai, J., and Arpo, K. 2010. Encapsulation of ascorbyl palmitate in chitosan nanoparticles by oil-in-water emulsion and ionic gelation processes. Colloids and Surfaces B: Biointerfaces, 76: 292-297.

Xynias, I.N., Mylonas, I., Korpetis, E.G., Ninou, E., Tsaballa, A., Avdikos, I.D., and Mavromatis, A.G. 2020. Durum wheat breeding in the Mediterranean region: Current status and future prospects. Agronomy, 10: 432.

Zhang, Q., Du, Y., Yu, M., Ren, L., Guo, Y., Li, Q., Yin, M., Li, X., and Chen, F. 2022. Controlled release of dinotefuran with temperature/pH-responsive chitosan-gelatin microspheres to reduce leaching risk during application. Carbohydrate Polymers 277: 1-11.

Ziaee, M., Moharramipour, S., and Mohsenifar, A. 2014. MA-chitosan nanogel loaded with *Cuminum cyminum* essential oil for efficient management of two stored product beetle pests. Journal of Pest Science 87: 691-699.
