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The effect of repellency of powder formulations from *Artemisia sieberi* (Asteraceae) essential oil on *Sitophilus oryzae* (Col.: Curculionidae)

E. Shahinfar[1], A. Heidari[2], S. Imani[1], A. Ahadiyat[1], M. Negahban[3]

1- Department of Plant Protection, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2- Associate Professor of Pesticide Research Department, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
3- Assistant Professor of Pesticide Research Department, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

Abstract

Nowadays, extensive research is being done on plant compounds to find safer and low-risk alternative for controlling pests. In this study, two formulations, containing *Artemisia sieberi* Besser essential oil based on biodegradable polymers were prepared as powders to control the rice weevil, *Sitophilus oryzae*. These formulations were different in the amounts and types of polymers and surfactants. The repellency effect of these formulations and *A. sieberi* oil was compared at two sublethal concentrations of 5 and 10 µI/I air. The surface morphology of powders was also studied by the transmission electron microscope (TEM). The average size of particles was measured about 41.19 and 37.86 nm for formulations 1 and 2, respectively, using the software Image j. The result shows that, after 96 hours, the percentage of repellency in essential oil, formulation 1 and formulation 2 for 5 ppm concentration is calculated 45%, 65% and 70% respectively, and for 10 ppm concentration it is calculated 55%, 70% and 75% respectively. Also, the repellency effects of normal essential oil and the formulations prepared at different times showed that with increasing the concentration of all compounds, the amount of repellency increases. Based on the results, to maintain the biological activity of essential oils and prevent the evaporation of their constituents, it is necessary to formulate essential oils, therefore encapsulation of plant essential oils as a safe pesticide with gradual release and maintenance of fumigant toxicity can be considered in integrated pest management.

Keywords: Rice weevil, encapsulation, repellency.

* Corresponding Author, E-mail: *ahmad.heidari419@gmail.com*
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1.Introduction

Due to the quantitative losses, they cause, insects are one of the basic problems of stored grains worldwide (Fileds 2006). The rice weevil, *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae), is one of the most important pests of stored products, distributed throughout the world by international communications. This insect attacks many cereals, but rice is the main target. Depending on the product, the damage will be up to 50% by weight (Hosseini *et al.* 2008; Singh 2017). Entomologists worldwide have long been looking for efficient control of stored grain pests. For several years, synthetic chemical pesticides have been used to control the stored grain pests (Salem *et al*. 2007). Increasing resistance of storage pests to conventional chemical pesticides, such as methyl bromide and phosphine, in the storage room and the problems of pesticide residues in food, water and environmental pollution, plant contamination and toxic effects on mammals and the environment, the need to use newer and safer compositions for the environment and humans have made it necessary (Boyer *et al.* 2012; Nobari *et al.* 2014). The tendency to use natural compounds with environmental compatibility and desired pesticide characterization is expanding, so for this reason, nowadays a large amount of researches are concentrated on biorational pesticides (Isman *et al.* 2011). Plants are a rich source of defensive chemicals which could be extracted from different plant parts, such as flowers, tubers, leaves, fruits, branches and roots (Wink *et al.* 1998; Maia and Moore 2011). These materials may exhibit insecticidal properties, repellency, attractiveness, anti-nutritional, and the effect of growth regulators on insects (Champagne *et al.* 1992). *Artemisia sieberi* Besser belongs to the family Asteraceae and the Iranian indigenous (Mir Heidar 1994; Bagheri 2007). There are 400 species of *Artemisia* in the world, which about 34 species grown in Iran (Rabie *et al*. 2006). The genus Artemisia has numerous medicinal qualities for various treatments, including anti-coughing properties, antispasmodic properties, anesthetic, anti-parasite, analgesic and headache (Mir Heidar 1994; Shafizadeh 2002), antioxidant (anti-cancer), antidiabetic, anti-fungal as well as insecticidal properties (Shahbazi and Arshadi 2018). Artemisia sieberi is the most valuable species in terms of livestock nutrition and resistant to desert and semi-desert areas in Iran. It is important in protecting the environment, especially for soil erosion (Kargar et al. 2010).

The presence of volatile compounds with high evaporation rate and degradation against sunlight are the constraints for using essential oils. Therefore, one way to increase the stability and performance improvement of these plant compounds is to formulate them (Majeed et al. 2015). The choice of formulation depends on a variety of factors, including bioavailability, physical and chemical factors of the essential oil, the mode of action and the type of product (Negahban et al. 2013). Due to its lipophilic and low solubility in the water, plant essential oils require a transition system and their protection for greater efficacy using the controlled release formulation (Donsi *et al.* 2011). The encapsulation technology reduces the amount of active ingredient loss and, by a coating of active ingredients, protects them from environmental factors, such as temperature, light, and humidity. In this formulation the release of the active ingredient is gradual, as a result, it cuts feeding and ultimately leads to death of insect (Moretti *et al.* 2002).

Shahinfar *et al.* (2021), reported the lethal effects of nanopowders prepared from *A. sieberi* essential oil on adult *Sitophilus oryzae* insects. Their results showed that the LC_{50} content of pure essential oil was 11.33 ppm, but increased after formulation.

The production of encapsulated powder formulation contains plant essential oils as a safe and healthy biorational pesticide with high performance. Due to its significant toxic effects on pests, new and safe technologies can be obtained instead of chemical pesticides and have the least harmful effect on human and environments. This is a completely natural formulation based on industrial materials at an affordable price. In this research, an herbal source and environmentally friendly pesticide was prepared as powders and pellet to control of the rice weevil.

2. Materials and Methods

2.1. Insect Rearing. Adult rice weevils were collected from rice storage depots and grown in pest control laboratory at the Iranian Research Institute of Plant Protection, Tehran, Iran, at 27 ± 1 °C, 70±5 % RH in darkness condition in the germinator system on the rice cultivar 'Shiroudi'. For obtaining identical insects sample after four generations (growth period of insects, 28–30 days), the adult insects of 1 to 3 days (in both sexes) were used for initial tests and final experiments.

2.2. Preparation of Essential Oil. Artemisia sieberi essential oil used in this research was purchased from Barij Essence Pharmaceutical Co. (Kashan, Iran).

2.3. GC-MS analysis. The essential oil of *A. sieberi* was analyzed by GC-MS (Agilent 7890A) coupled with a mass spectrometer (Agilent 5975C) with a strong library for identification of isolated substances, located at the Research Institute of Forests and Rangelands, Tehran, Iran. The GC-MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm) with the oven temperature which was programmed as follows: the initial temperature of 60 °C was immediately increased to 220 °C at a rate of 3°C/min, subsequently the temperature was increased to 240 °C at 20 °C/min and held at this temperature for three mines. The injector and transfer line temperature were 260 and 280°C, respectively; carrier gas was helium with a linear velocity of 30.6 cm/s; split ratio 1:100, ionization energy 70 eV, scan time 1 s, mass range 40–340 a.m.u.

2.4. Formulations

2.4.1. Formulation 1. For 10 % (w/w) formulation, the amount of 10 gr of A. sieberi essential oil was mixed with 5 gr of Glycerin, 5 gr of Polyethylene glycol and 5 gr of tween 80 (Polyoxyethlene (20) sorbitan monooleate), and with the help of a mechanical mixer at the speed of 400–1200 rpm (model: Hei-TORQUE Value 400 Basis). Then, 75 gr of starch were added. Finally, to prevent adhesion and absorption of moisture, 4 gr of sodium sulfate (Na_2SO_4) were added to the powder (shahinfar *et al.*, 2021).

2.4.2. Formulation 2. For 10% (w/w) formulation needs 10 gr of A. sieberi essential oil, 10 gr of Polyethylene glycol and 1 gr of coconut oil (coconut fatty acid diethanolamide) as a surfactant were added and mixed for 15 minutes with a mechanical mixer at the rate of 500–1000 rpm mixed. In another container, 55 gr of starch, 10 gr of Maltodextrin, 5 gr of Na_2SO_4 and a specific amount of Xanthan gum were mixed. The essential oil solution was then added to the powder containing starch drop by drop. The final formulation was powdered (shahinfar *et al.*, 2021).

2.5. Survey of powder formulations surface morphology and nanoparticle size

The transmission electron microscope (TEM) was used for observation the surface and morphology of powder formulations and the mean particle size was calculated by software Image j.

2.6. Bioassay Tests. The experiments were carried out at the Bioassay Laboratory of the Pesticide Research Department at the Iranian Research Institute of Plant Protection and all experiments were conducted in a germinator at 28 ± 1 °C and 70 ± 5% RH in continuous darkness.

2.7. Repellency Bioassay

The experiment was conducted as described by Shakarami *et al*. (2004) with some modifications. The repellency bioassay test chambers were consisted of two plastic chambers (65 ml volume) joined to either side of a central main chamber with the same size by a small tubing (2 cm long and 5 mm diameter). So that insects could easily move from the middle chamber to the side chambers through the clear tubes. In this experiment, 1gr of rice used for water control and 5 and 10 ppm of the essential oil and formulations prepared with 1gr of rice were used in the treatment. Although, for concentrations of 5 and 10 ppm of the active ingredient from formulations respectively, the amount of 0.042 and 0.083 gr of powder were weighted and were poured into the treatment container with nutrient (1g of rice). Subsequently, 10 adult insects with 1 to 3 days old after 24 hours of starvation were released into the middle chamber as the basal container. The lids were open during the test and covered with mesh. The number of insects on each side of the container was counted after 1, 3, 6, 12, 24, 48, 72 and 96 hours. The experiment was performed in four replications.

2.8. Data Analysis. ercentage repellency was calculated based on following formula (Liu *et al*. 2006):

Repellent Index (RI) % $= \frac{C-T}{G} \times 100$

C: Number of insects in control container

T: Number of insects per treatment

G: Total number of insects

The results were analyzed by using SPSS 22. For this reason, $Arcsine\sqrt{x/100}$ used as normal repellency index, and one-way analysis of variance (abbreviated one-way ANOVA), is a technique that can be used to compare means of two or more samples, was used for grouping. If the result of grouping became meaningful then, the means were compared by Tukey test at the 5% level.

3. Results

3.1. Chemical composition of *A. sieberi* essential oil. The GC-MS results of essential oil analysis for *A. sieberi* showed that the four major components of this essential oil were Cis-thujone (38.20%), Santolinyl acetate (23.77%), Trans-thujone (11.31 %) and Camphor (6.30 %) (Table 1). The GC/ MS profile of *A. sieberi* essential oil is presented in Fig 1.

Table 1 GC-MS analysis of *Artemisia sieberi* essential oil

NO.	Compound	Retention Time (min)	Percentage	Kovats Index (KI)
1	α-pinene	1.94	0.70	94
2	Camphene	2.04	4.27	950
3	yomogi alcohol	2.13	2.17	1003
4	p-cymene	2.34	1.04	1026
5	1,8-cineol	2.39	3.16	1037
6	Artemisia alcohol	2.52	1.55	1085
7	Cis-thujone	2.72	38.20	1105
8	Trans- thujone	2.77	11.31	1117
9	Comphor	2.81	6.30	1141
10	Borneol	2.89	2.62	1165
11	Santolinyl acetate	2.95	23.77	1171
12	Terpinen-4-ol	3.03	0.88	1180
13	p-cymen-8-ol	3.05	0.57	1182
14	verbenone	3.18	1.14	1202
15	Cis-chrysanthenyl acetate	3.27	1.14	1268
16	Bornyl acetate	3.4	0.69	1281
17	Other compounds	-	0.49	-
	Total		100	

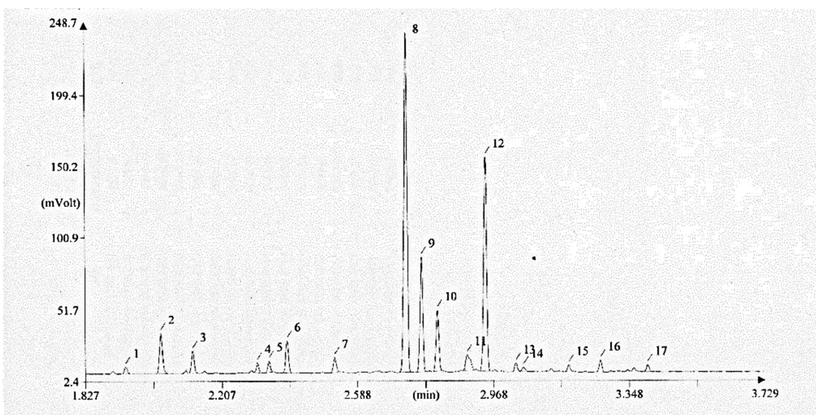


Fig1 GC/ MS profile of *Artemisia sieberi* essential oil

3.2. Survey of powder formulations surface morphology and nanoparticle size. TEM images (as can be seen from Fig. 2) show nucleus and wall structure of nanopowders. The mean of particle for formulations 1 and 2 were measured 41.19 and 37.86 nm, respectively.

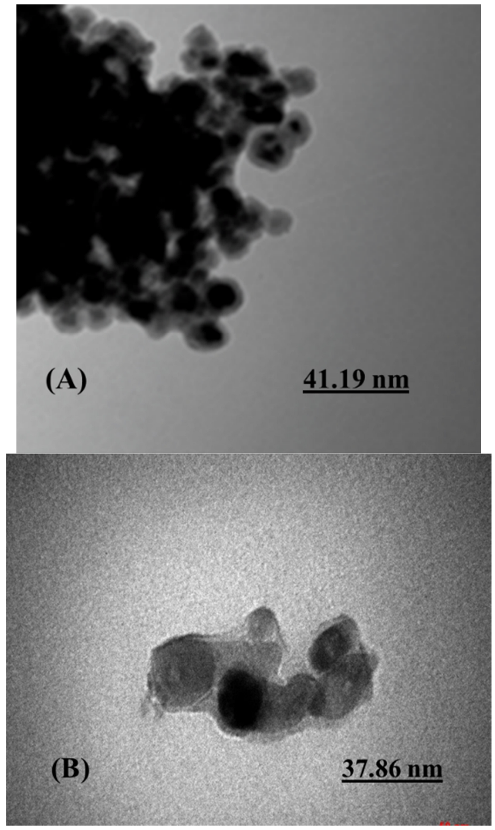


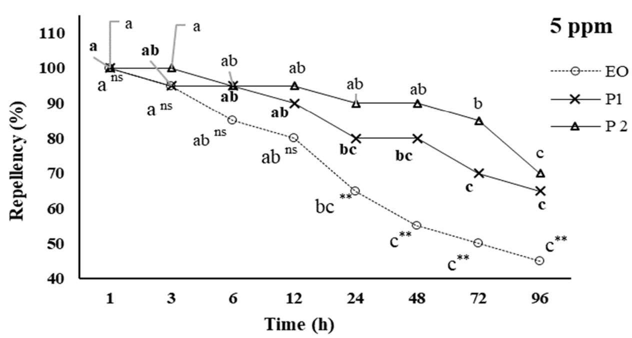
Fig 2 Transmission microscope images of nanopowders core structure from *Artemisia sieberi* oil (A: formulation 1 and B: formulation 2)

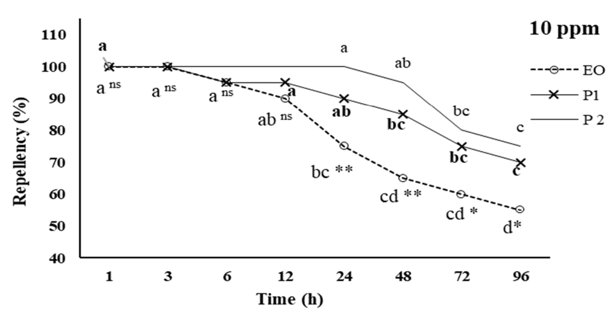
3.3. Repellency Bioassay

The repellency effect of pure essential oil and formulations containing *A. sieberi* oil was studied at two sublethal concentrations of 5 and 10 µI/I air. The results showed that a concentration of 5 ppm, pure essential oil was 100% replant in the first hour, but over time, the repellency rate was decreased, so that after 96 hours, the repellency rate was 45%. However, in the first 12 hours of Formulation 1, the repellency rate was more than 90%, but then gradually began to decrease, and finally reached 65% after 96 hours. In formulation 2, in the early hours, it was 100% replant and retained its 90% repellency for up to 48 hours, and finally, after 96 hours, the repellency rate was 70%. At a concentration of 10 ppm of pure essential oil, the highest repellency rate was observed in the first 3 hours, but it was decreased over time, and finally reached 55% after 96 hours. Formulation of powder 1 at 10 ppm concentration was 100% repellant for the first hour and then decreased until it reached 70% repellency after 96 hours. The powder formulation 2 maintained its repellency at 10 ppm for 24 hours up to 100% and then began to decrease (Table 2) (Fig.3).

Table 2 The repellency of pure essential oil and powder formulations containing *Artemisia sieberi* essential oil on *Sitophilus oryzae* adults

concentration (ppm)	time(h)	Compounds Repellency (%)		
		Essential Oil	**formulation 1**	**formulation 2**
5	1	100± 0.00	100± 0.00	100± 0.00
	3	95± 2.04	95± 2.89	100± 0.00
	6	85± 5.00	95± 3.54	95± 2.89
	12	80± 8.16	90± 2.89	95± 3.54
	24	65± 5.40	80± 4.56	90± 2.89
	48	55± 5.77	80± 4.56	90± 2.89
	72	50± 5.77	70± 5.77	85± 2.89
	96	45± 5.77	65± 5.00	70± 2.89
10	1	100 ± 0.00	100 ± 0.00	100 ± 0.00
	3	100 ± 0.00	100 ± 0.00	100 ± 0.00
	6	95 ± 2.04	95 ± 3.54	100 ± 0.00
	12	90±3.54	95 ± 2.04	100 ± 0.00
	24	75± 5.40	90 ± 2.89	100 ± 0.00
	48	65± 5.40	85 ± 2.04	95± 2.04
	72	60± 4.08	75 ± 5.40	80± 4.56
	96	55± 5.77	70± 5.77	75± 5.40





Eo: Essential Oil, P_1: $Powder_1$, P_2: $Powder_2$
Means followed by same small letters are not significantly different (Tukeys test, $p< 0.05$)
ns: not significantly
**: significantly in level 0.01
*: significantly in level 0.05

Fig 3 Comparison of repellen activity of *Artemisia sieberi* essential oil and nanopowder of oil on *Sitophilus oryzae* adults

4. Discussion

A decrease in food quality would be important for controlling *S. oryzae*. In this research, multiple eco-friendly formulations have been used which reduce essential oils limitation in insect pest control. It should be noted that there is no data on the impact of formulations containing essential plant oils on *S. oryzae*. A new method for producing nanopowders has been employed in the present study. Adjuvant formulated products and essential oil used in these formulations are fully environmentally friendly. With this technique, the generated nanopowders can be used in storage houses to preserve food from pest damage. The mentioned nanopowders have been produced from nanoparticle of *A. sieberi* as active ingredient.

Insecticidal compounds of many essential oils are monoterpenoids. Camphor, camphene, 1.8-cineol, α-pinene α, linalool, methyl acetate, limonene, menthone, graniol, citral, citronellal, thymol, carvacrol, eugenol and trans-anethol are examples of pesticide compounds of plant essential oils (Isman and Machial 2006; Philips *et al*. 2010). In addition, monoterpenoids are highly regarded as control agents and are actually toxic to insects (Moharramipour and Negahban 2014). In this study, *Artemisia* essential oil had different monoterpenoids with high evaporation rate. Due to their high volatility, they had fumigant activity that might be of importance for controlling stored-product insects. Bayramzadeh *et al*. (2019) showed that two essential oils, including *Cuminum cyminum* Linnaeus and *Lavandula angustifolia* Miller, were nanocapsulated by solvent evaporation emion method and their fumigant toxicity was investigated against *Tribolium castaneum* Herbst and *Sitophilus granarius* Linnaeus. Their results suggested that the content of essential oils could influence encapsulation efficiencies.

In this study, the repellency effect of normal essential oil of *A. sieberi* and its various formulations were studied in different times. It can be clearly seen that the percentage of the repellency is increased by increasing concentration for all compounds.

Negahban (2012) showed that *Artemisia* nanoencapsulated essential oils at different concentrations increased repellency over time while normal essential oils loss it repellency gradually by time.

Jamal *et al.* (2012) evaluated the repellency of nanoencapsulated *Carum copticum* essential oils on *Plutella xylostella* was longer than normal essential oil. Nuchuchua *et al*. (2009) conducted a study on the repellency effect of different essential oils nanoemulsion on *Aedes aegypti* (L.) mosquitoes and it was shown that this formulation had better physical stability and longer repel activity. Negahban *et al*. (2013) showed that the repellency effect of normal essential oil with concentration of 1.9 ppm on *Plutella xylostella* reached its highest level of 80% within six hours, but after 24 hours it was just 62% repellent. However, the repellency effect of nanocapsulated formulation was increased by time and reached 100% after 24 hours.

The results suggest that the process of encapsulating plant essential oils releases the active ingredient in a controlled manner and maintains its toxicity for a longer period of time.

5. Conclusions

At the present, the introduction of low risk, effective and low potentials resistance insecticide to control the pest are felt more than ever. Essential oils and plant extracts with natural origin can be effective in controlling pests in the warehouses by solving commercialization problems and their formulation issues. Also, considering the recent trend of society to use safe food with minimal synthetic pesticide remains reveals the need for bio-based pesticides production, while also being effective and low-risk for the environment. Plant essential oil compounds mainly contain monoterpenoids, and these compounds are highly volatile and have little durability in the environment. Therefore, the durability of the essential oil decreases after a short time on the insects. The use of encapsulating technology by creating polymer coating around the particles of essential oil is a novel and suitable method for preserving the essential oils from fast oxidation, which lead to maintain its insecticide effect and longer durability. The type of polymer selected can vary in the amount of essential oil released from the capsules. Therefore, with the proper choice of formulation in the pest management programs, rapid release formulation can be used to have impacts on severe contamination of stored products. The formulation with gradual release can also be used to prevent pest attack.

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دانشگاه آزاد اسلامی، واحد اراک
شاپا ۴۶۶۸-۲۰۰۸
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فصلنامه تخصصی تحقیقات حشره‌شناسی
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اثرات دورکنندگی فرمولاسیون های پودری حاوی اسانس درمنه *Artemisia sieberi* (Asteraceae) علیه شپشه برنج *Sitophilus oryzae* (Col.: Curculionidae)

الهام شاهین فر[۱]، احمد حیدری[۲]، سهراب ایمانی[۱]، علی احدیت[۱] و مریم نگهبان[۳]*

۱- گروه گیاهپزشکی، واحد علوم وتحقیقات، دانشگاه آزاداسلامی، تهران، ایران
۲- دانشیار بخش تحقیقات آفتکش ها، موسسه تحقیقات گیاه پزشکی کشور، تهران، ایران
۳- استادیار بخش تحقیقات آفتکش ها، موسسه تحقیقات گیاه پزشکی کشور، تهران، ایران

چکیده

امروزه تحقیقات گسترده‌ای بر روی ترکیبات گیاهی به منظور دستیابی به سموم جایگزین ایمن‌تر و کم‌خطرتر برای کنترل آفت صورت می‌پذیرد.

در این مطالعه، دو فرمولاسیون حاوی اسانس *Artemisia sieberi* Besser بر پایه پلیمرهای تجزیه پذیر به صورت پودر برای کنترل شپشک برنج *Sitophilus oryzae* تهیه شد. فرمولاسیون های تهیه شده از نظر میزان و نوع پلیمرها و سورفاکتانت ها متفاوت بودند. اثرات دورکنندگی این فرمولاسیون ها و اسانس خالص در دو غلظت زیر کشندگی µl/l air ۵ و ۱۰ مقایسه شد. مورفولوژی سطح ذرات کپسول ها توسط میکروسکوپ الکترونی عبوری (TEM) مورد مطالعه قرار گرفت. میانگین اندازه ذرات به ترتیب برای فرمولاسیون ۱ و ۲ با استفاده از نرم افزار Image j در حدود ۱۹/۴۱ و ۸۶/۳۷ نانومتر اندازه گیری شد. نتایج نشان داد، پس از گذشت ۹۶ ساعت ، درصد دورکنندگی در اسانس درمنه ، فرمولاسیون پودری ۱ و ۲ برای غلظت ppm ۵ به ترتیب ٪۴۵ ، ٪۶۵ و ٪۷۰ و برای غلظت ppm ۱۰، به ترتیب ٪۵۵ ، ٪۷۰ و ٪ ۷۵ محاسبه شد. همچنین اثرات دورکنندگی اسانس خالص و فرمولاسیون های تهیه شده در زمان های مختلف نشان داد با افزایش غلظت درهمه ترکیبات ، میزان دورکنندگی افزایش می یابد. براساس نتایج بدست آمده ، برای حفظ فعالیت بیولوژیکی اسانس ها و جلوگیری از تبخیر مواد تشکیل دهنده آن‌ها، فرموله کردن اسانس‌ها امری ضروری است ، بنابراین کپسوله کردن اسانس های گیاهی به عنوان یک آفتکش با رهایش تدریجی و حفظ سمیت تنفسی می تواند در مدیریت تلفیقی آفات در نظر گرفته شود.

واژه‌های کلیدی: شپشک برنج، کپسوله کردن ، دور کنندگی

* نویسنده رابط، پست الکترونیکی: ahmad.heidari419@gmail.com

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