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Synthesis, Characterization, and *in Vitro* Antifungal Activity of Solid Lipid Nanoparticles Containing *Mentha×piperita* L. Essential Oil

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ABSTRACT

The use of essential oils and new drug delivery systems have been considered two approaches for controlling plant pathogenic fungi. This study aimed to synthesize, characterize, and evaluate the antifungal activity of Solid Lipid Nanoparticles (SLNs) incorporating *Mentha×Piperita* L. Essential oil (MPE) compared to the free MPE. In the present study, the formulations of SLNs incorporating MPE (MPE-SLNs) were synthesized by high-shear homogenization and ultrasound method, and they were assessed by Z-average diameter, particle size distribution, Zeta potential, leakage stability during 6 months of storage, encapsulation efficacy, and morphological properties of the SLN formulations. The results indicated that the particle size of MPE-SLN formulations was 155.5±4.7 nm with a PDI of 0.156±0.012, a Zeta potential of -15.93±0.87 mV, and encapsulation efficacy of about 88±0.88%. They were physically stable for 6 months of storage. The results also showed that the *in vitro* minimum inhibition concentration for MPE on the fungal microorganisms, *Rhizoctonia solani* and *Rhizopus stolonifer*, were 2,000 and 1,000 ppm, respectively, and for MPE-SLNs it was 1,000 and 750 ppm, respectively. Therefore, the antifungal activity of MPE-SLNs was more significant than MPE, and none of the fungi were susceptible to essential oil-free SLNs. Based on the results, MPE-SLNs can be used for the safe preservation of a wide array of foods and agricultural products.

Keywords: Food preservation, Fungal pathogens, *Rhizoctonia solani*, *Rhizopus stolonifer*.

INTRODUCTION

Fungal pathogens cause significant damage to pre- and postharvest agricultural products; and reducing these losses with the aim of increasing food security and preventing capital losses is one of the most important challenges for farmers and consumers. Essential oils are promising bioresources for the management of fungal pathogens, as they are safe for human and environmental health, easy to acquire from plants, and cost-effective to produce (Plavšić *et al.*, 2017; França *et al.*, 2018). Numerous studies have mentioned the biological properties of essential oils, especially their antifungal properties (Tang *et al.*, 2018; Yusoff *et al.*, 2018; Wan *et al.*, 2019; Vakili-Ghartavol *et al.*, 2022). However, poor water solubility, degradation or evaporation in adverse environmental conditions such as oxygen, light, humidity, and pH limit the use of essential oils (Donsì

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et al., 2011; Shetta *et al.*, 2019; Rajkumar *et al.*, 2020; Laein *et al.*, 2022). One of the promising technologies for overcoming these limitations is nano-encapsulation of essential oils (Ghodrati *et al.*, 2019; Shetta *et al.*, 2019; Rajkumar *et al.*, 2020).Therefore, the use of essential oils and new drug delivery systems have been considered the two important approaches for controlling plant pathogenic fungi (Shetta *et al.*, 2019; de Oliveira *et al.*, 2020).

One of the techniques of nano-encapsulation is the use of Solid Lipid Nanoparticles (SLNs), which were introduced in1990 (Katarija and Prajapati, 2013). SLNs are colloidal carriers with a size of approximately 50 to 1,000 nm and are composed of physiological lipids that are solid at ambient temperature, and are advantageous over drugs with poor water solubility and, consequently, low bioavailability (Katarija and Prajapati, 2013; Laein *et al.*, 2022). Other unique features of SLNs include large surface area, small size, high effectiveness, high drug loading, and the possibility to develop new therapeutics that could be used for drug targeting (Cavalli *et al.*, 1993; Sarangi and Padhi, 2016).

Tween 80 is composed of poly ethoxylated sorbitan and oleic acid, and the hydrophilic groups in this composition are polyethers, which are polymerized ethylene oxides (Umoruddin *et al.*, 2019). According to the Food and Drug Administration (FDA), Tween 80 is safely used as a food additive (Anonymous, 2021), and also acts as a surfactant and lubricant in food products, particularly in ice cream, where it is added up to a concentration of 0.5% to make the ice cream softer and increase its resistance to melting (Lu *et al.*, 2014).

Different essential oils and chemical compounds derived from plants were encapsulated in SLNs such as Xanthan gum (Zambrano-Zaragoza *et al.*, 2013), *Zataria multiflora* (Moghimipour and Handali, 2012; Moghimipour *et al*., 2013; Nasseri *et al*., 2016; Kelidari et al., 2021), cinnamaldehyde, eugenol, and thymol (McDaniel *et al.*, 2019), copaiba oil and allantoin (Svetlichny *et al.*, 2015), *Eugenia caryophyllata* (Fazly Bazzaz *et al.*, 2018), hesperetin (Fathi *et al.*, 2013), *Artemisia arborescens*(Lai *et al.*, 2006; Lai *et al.*, 2007), frankincense and myrrh (Shi *et al.*, 2012), rosemary (Montenegro *et al.*, 2017), Yuxingcao (Zhao *et al.*, 2017), *Nigella sativa* L. (Al-Haj *et al.*, 2010), safranal (Khameneh *et al.*, 2015) for various purposes. To the best of our knowledge, no studies have been done on synthesizing SLNs incorporating *Mentha×Piperita* L. Essential oil (MPE) and their influence against fungal pathogens.

Mentha×piperita L. is a herbaceous plant from the Lamiaceae family and a hybrid of a cross between water mint and spearmint, originating from the Mediterranean regions (Nyegue *et al*., 2014). The extractions and essential oils derived from this plant have several traditional uses, such as the treatment of stomach disorders, muscle pains, and tooth ache, etc. (Shah and Mello, 2004; Vakili-Ghartavol *et al*., 2022). Several studies have focused on antifungal activity of MPE (Chraibi *et al.*, 2017; França *et al.*, 2018; Desam *et al.*, 2019).

The aim of the present study was to nano-encapsulate MPE in SLNs in order to enhance its physical properties, protect essential oil components, and improve its antifungal activities for food applications. The antifungal activity of MPE-SLN formulations was compared with that of the free essential oil encapsulations and pure essential oils against *Rhizopus stolonifer*, *Penicillium expansum*, and *Rhizoctoria solani* AG4-HG II.

MATERIALS AND METHODS

Essential Oil Analysis

Identification and quantification of *Mentha×piperita* L. essential oil was performed by Gas Chromatography-Mass Spectrometry (GC-MS) (Vakili-Ghartavol *et al.*, 2022) with a comparison of their mass spectra and retention indices with those

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authentic samples, computer library (NIST14N.L), and those given in the literature (Adams, 2007). Briefly, the GC-MS spectrometry analyses were performed using a Finnigan-Thermo Trace DSQ Mass Spectrometer system equipped with an HP-5MS fused silica column (30 m×0.25 mm id, film thickness 0.32 μm; J and W Scientific), using helium as carrier gas at a linear velocity of 1 mL min^{-1}. GC oven temperature was raised from 60 to 220°C at a rate of 3°C min^{-1}, the transfer line temperature was 250°C, the split ratio was 1:100, and Electron impact MS was 70eV.

Fungal Samples

The fungal strains used in this research, namely, *Rhizopus stolonifer*, *Penicillium expansum*, and *Rhizoctonia solani* AG4-HG, were obtained from the Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, in Iran. The identification of *R. solani* AG4-HG II was previously investigated by Pourmahdi and Taheri (2015), and the identification of other strains was morphologically done by light microscopy. Then, these strains were kept on Potato Dextrose Agar (PDA, Zona Industriale 64026, and Roseto Degli Abruzzi, Italy).

SLN Synthesis

MPE-SLNs and SLN formulations without MPE were synthesized using high-shear homogenization and ultrasound methods, previously described by Fazly Bazzaz *et al.*(2018), with slight modifications. Briefly, the lipid phase (Precirol® ATO 5, Gattefossé, France, 5% as a lipid, and Tween 80, Sigma, Germany, 2.5% as a surfactant) and the aqueous phase (double-distilled water up to 100%) were separately placed inside a Water Bath at 70-75°C. At the end of the melting process of the lipid, the MPE was added to the lipid phase to prevent the evaporation of the essential oil. After the components of the lipid phase were quickly mixed together, the hot aqueous phase was added to the melted lipid phase in a Water Bath at the same temperature. Then, they were mixed together and homogenized by a Diax 900 homogenizer (Heidolph, Germany) for 4.5 minutes. The resulting emulsion was ultrasonicated by a probe sonicator (Bransonic, USA). The sonication was performed in 5 cycles with 60 seconds of sonication separated by intervals of 15 seconds. Samples were then cooled to room temperature and SLN solutions were obtained. SLN formulations without MPE were also prepared by the same methods (Table 1).

Size, Polydispersity Index, and Zeta Potential Measurements

Z-average diameter (nm), Polydispersity Index (PDI), and Zeta potential of the MPE-SLN formulations were evaluated by nanoparticle analyzer based on the Dynamic Light Scattering (DLS) method (ZetaSizer Nano-ZS; Malvern Instruments Ltd., United Kingdom) (Montenegro *et al.*, 2017).

Stability Tests

SLN formulations were stored in 2 mL micro centrifuge tubes covered with aluminum foil. Then, they were kept in the refrigerator at 4°C for 6 months. Z-average diameter (nm), Polydispersity Index (PDI), and Zeta potential of the samples were evaluated at fixed intervals (24 hours, 3 months, and 6 months after their synthesis).

Encapsulation Efficiency (EE%)

The standard curve of menthol, the dominant component of MPE as an index, was drawn using the average area under the curve obtained from GC-MS at concentrations of 2, 4, 6, 8, 10, 25, 50, and 100 μg mL^{-1} to get actual menthol concentration in SLN

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Table 1. Components of MPE-SLNs, Emulsion, and Reference SLN Formulations without MPE.[a]

Formulation	Component	% (wt/wt)
MPE-SLN	Precirol® ATO5	5
	Mentha× piperita L. essential oil	0.1
	Tween 80	2.5
	water	92.4
SLNs without MPE	Precirol® ATO5	5
	Tween 80	2.5
	Water	92.5

[a] SLNs and MPE indicate Solid Lipid Nanoparticles and *Mentha×Piperita* L. Essential oil.

formulations. Then, the entrapped menthol concentration in the MPE-SLN formulation was measured after purification by GC-MS. To purify, 500 µL of the MPE-SLN dispersion in an Amicon Ultra-15, PLHK Ultracel-PL Membrane, 100 kDa, Millipore was centrifuged at 10,000 rpm for 30 minutes, then, we prepared a suitable dilution with chloroform: methanol (2:1 v/v) for GC-MS analysis (an injection of one µL) to get the concentration of encapsulated menthol in MPE-SLN formulations. Then, the encapsulation efficiency was determined by equation 1 (Shah and Mello, 2004; Nasseri *et al.*, 2016).The Encapsulation Efficacy (EE%) equation was used as follows:

$$EE\% = \frac{Actual\ Menthol\ concentration\ in\ sample}{input\ Menthol\ concentration} \times 100$$

Differential Scanning Calorimetry (DSC)

Mettler DSC 822e (Mettler Toledo, GieBen, Germany) was used for DSC studies. Five mg of samples were weighed in aluminum oxide pans, sealed, and analyzed at thermal conditions of a range of 20 to 220°C at a heating rate of 10°C min^{-1} under a nitrogen atmosphere (80 mL min^{-1}), and determined the melting point of the samples (Khameneh *et al.*, 2015).

Transmission Electron Microscopy (TEM)

For evaluation of the SLN morphology properties with transmission electron microscopy (TEM, CEM 902A; Zeiss, Oberkochen, Germany), the SLNs were diluted 100 times with distilled water and coated on a carbon-coated copper grid for 1 minute, the excess water was wiped off with filter paper. After that, 2% uranyl acetate in 20 µL water was placed on sample and, after 1 min, were wiped off by another filter paper. The grid was dried at room temperature. After drying, the sample was observed by Transmission Electron Microscopy (TEM) (Layegh *et al.*, 2013).

Antifungal Activity Investigation

Agar diffusion technique was used to study the antifungal activity of samples with different concentrations of 0, 250, 500, 750, 1,000, and 2,000 ppm against *Rhizopus stolonifer*, *Penicillium expansum*, and *Rhizoctonia solani* AG4-HG on PDA media (Vakili-Ghartavol *et al.*, 2022). Briefly, the MPE were dissolved in 5% Tween 80. The required amounts of the samples were added to individual Petri dishes containing 20 mL of PDA medium at 40°C. A 2 mm disc of mycelium of each strain was placed in the center of the Petri dish, incubated at 25±3°C, daily measured the mycelial growth, and calculated the Inhibition Percentage (IP) by equation 2. Also, the lowest concentration of the treatments in which no mycelium growth was observed was obtained as the minimum inhibitory concentration.

The Inhibition Percentage (IP%) equation used was as follows:

$$IP\% = \frac{(dc - dt)}{dc} \times 100$$

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Where, dc is the mycelium growth diameter in the control Petri dish and dt is the mycelium growth diameter in the treated Petri dish.

Statistical Analysis

ANOVA and Duncan's multiple range test (< 0.05) were used to analyze data by the Statistical Analysis System SPSS 24.0 software, graphs were prepared by Microsoft Excel 2013, and references were managed by EndNoteX8 software. The data were shown in three replicates and as mean ± standard deviation.

RESULTS

Chemical Compositions of MPE

MPE consisted of 15 compounds, of which menthol and menthone made up a total of 64.1% and were oxygenated monoterpenes (Table 2).

Characterizations of SLN Formulations

MPE-SLN formulations had a Z-average of 155.5±4.7 nm, a PDI of 0.156±0.012, and a Zeta potential of -15.93±0.87mV, their average particle size increased only slightly during 6 months of storage at 4˚C, while SLNs without MPE had a Z-average of 149.7±0.17 nm, a PDI of 0.142±0.01, and a Zeta potential of -10.9±0.17mV (Table 3).

Encapsulation Efficiency

The Encapsulation Efficiency (EE) of the MPE-SLN formulations was 88±0.88%, based on the dominant composition of the MEP, menthol, which was selected as the index component.

Differential Scanning Calorimetry (DSC)

DSC diagrams of MPE, bulk lipids (Precirol® ATO5), and MPE-SLN formulations are presented in Figure 1. Temperatures of 56, 171, and 58˚C were the melting for Precirol® ATO5, the MPE, and MPE-SLNs, respectively [Figure 1 (B, A, and D)]. The melting peak of formulations without MPE was similar to the formulations incorporating MPE [Figure 1 (C, 58˚C)]. MPE-SLN formulations had no

Table 2. Chemical components of *Mentha×piperita* L. essential oil.

No.	Components	Quantity (%)	Retention Time (RT)
1	o-Cymene	0.01	7.84
2	D-Limonene	0.1	7.95
3	Eucalyptol	0.4	8.06
4	Linalool	0.1	9.8
5	Isopulegol	0.2	11.25
6	l-Menthone	27.7	11.51
7	Benzofuran, 4,5,6,7-tetrahydro-3,6-dimethyl-	1	11.65
8	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, cis-	4.2	11.74
9	Cyclohexanol, 1-methyl-4-(1-methylethyl)-	5.5	11.82
10	d-Menthol	36.4	12.08
11	Pulegone	5.9	13.78
12	Carvone	2.2	13.94
13	Menthyl acetate	11.2	15.07
14	Caryophyllene	2.1	18.55
15	Caryophyllene oxide	1	22.64
16	Total	98.01	

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Table 3. Z-average diameter (nm), Polydispersity Index (PDI), and Zeta potential of the formulations calculated by DLS.

	SLNs without MPE	MPE-SLNs		
	After 24 hours	After 24 hours	After 3 months	After 6 months
Z-average diameter (nm)	149.7±0.17	155.5 ± 4.7	158 ± 0.8	158.1± 3.1
PDI (intensity)	0.142 ± 0.01	0.156 ± 0.012*	0.174 ± 0.01*	0.163 ± 0.1*
Zeta potential (mV)	-10.9± 0.17	-15.93 ± 0.87**	-14.4 ± 0.2**	-16.05 ± 1.5**

**and *: Values in the same row are significantly different (P< 0.01 and 0.05).

melting peak of MPE.

Transmission Electron Microscopy (TEM)

The morphological images of the nanoparticles of the MPE-SLN formulations were almost spherical and uniform with a size of about 50 to 300 nm, which was consistent with the data obtained from the particle size analysis (Figure 2).

In vitro Antifugal Activity

Mycelium growth of fungal pathogens treated with free MPE, formulations without MPE, and MPE-SLN formulations are shown in Figure 3. Each fungal strain showed different degrees of mycelium growth according to different concentrations of treatments. Comparing the average mycelium growth of the treated fungi compared to the control treatment, a significant decrease in the fungal colony diameter was observed when the concentration value was higher than 250 and 500 ppm for MPE-SLN and MPE (P< 0.01). The results also indicated that the *invitro* minimum inhibition concentration for the MPE-SLN formulations on the fungal microorganisms, *R. solani* and *R. stolonifer*, was a concentration value of 1000 ppm and 750 ppm , respectively, for the MPE, it was 2000 ppm and 1000 ppm (Table 4). Therefore, the results indicated that the antifungal activity of MPE-SLN formulations was significantly (P≤ 0.01) stronger than MPE alone. It inhibited the fungal mycelium growth at a lower concentration. The most sensitive and tolerant microorganisms to these treatments were *R. stolonifer* and *P. expansum*, respectively. Meanwhile, none of the microorganisms were sensitive to the SLN formulations without MPE (Table 4).

DISCUSSION

The findings of the qualitative-quantitative analysis of MPE were consistent with İşcan *et al.* (2002) and Kostik *et al.* (2015), who reported menthol as the major component of the essential oil. However, the MPE components showed significant variation from the previous report of Goudjil *et al.* (2016), who found carvone and limonene as the principal components. Such chemical composition changes in the essential oils may be due to the geographical origin of the plant, the time of harvest, developmental, environmental, and climate factors (Mehani *et al.*, 2015).

To overcome the problems related to the volatility of MPE and its efficient use as natural antifungals in the food industry, MPE was nano-encapsulated in SLNs (Montenegro *et al.*, 2017; Fazly Bazzaz *et al.*, 2018; Kelidari *et al.*, 2021), with an encapsulation efficacy of 88±0.88%. As reported in the literature (Mokarizadeh *et al.*, 2017; Fazly Bazzaz *et al.*, 2018), this high encapsulation percentage was due

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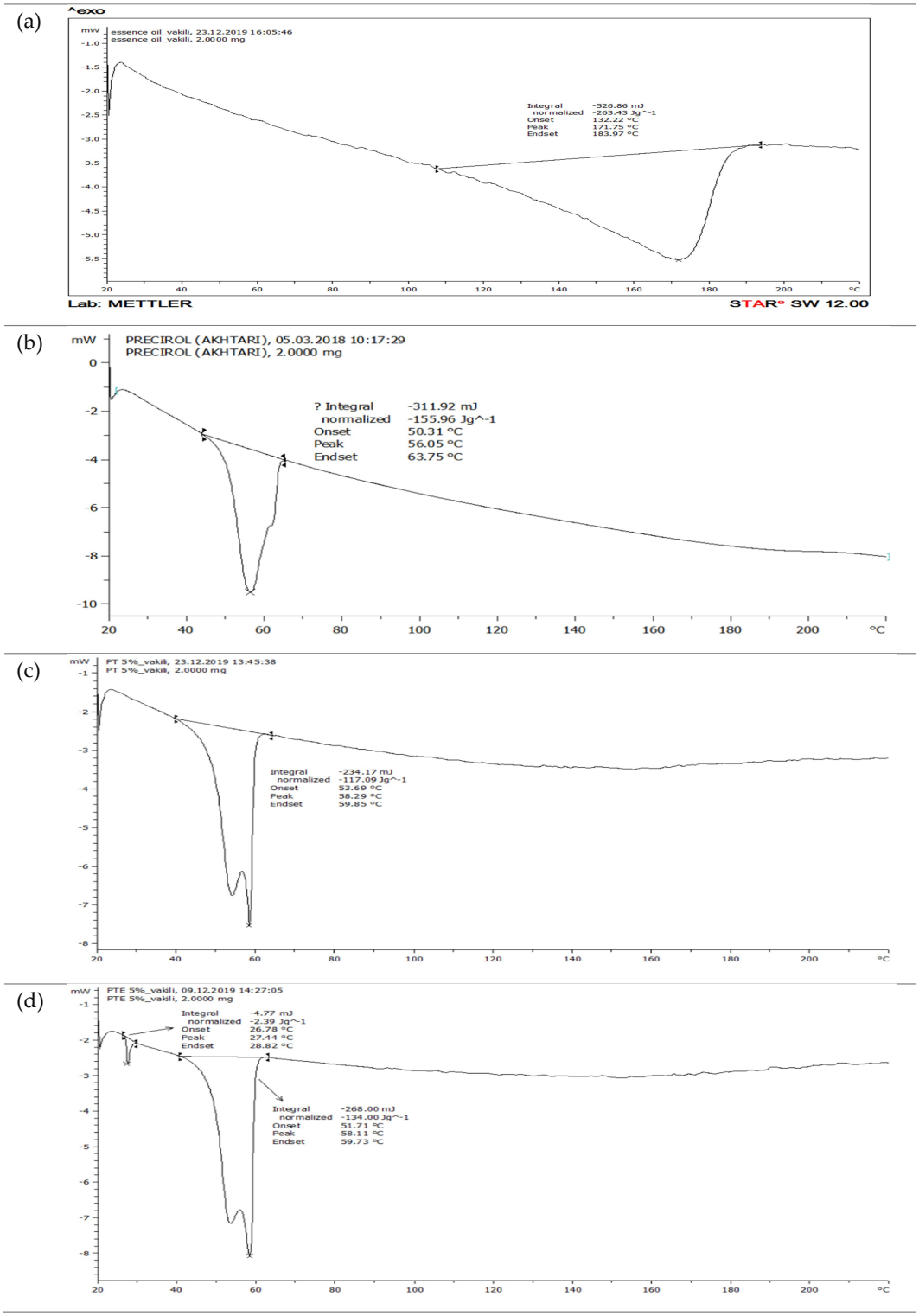

Figure 1. Differential Scanning Calorimetry (DSC) thermograms: (a) MPE, (b) Precirol® ATO5 bulk, (c) SLN formulations without MPE, and (d) MPE-SLN formulations prepared by high-shear homogenization and ultra sound technique. 2 mg of each sample was used in each run.

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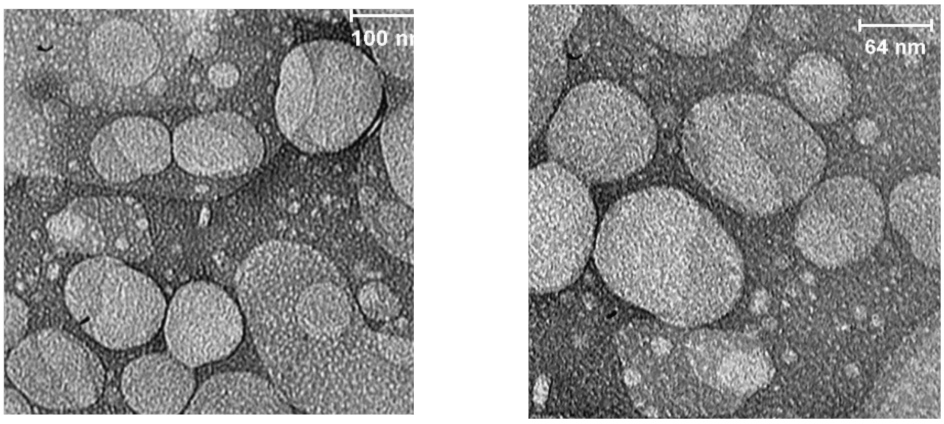

(a) (b)

Figure 2. Electron microscopy images of TEM from MPE-SLN formulations, (a) A size of 100 nm, and (b) A size of 64 nm.

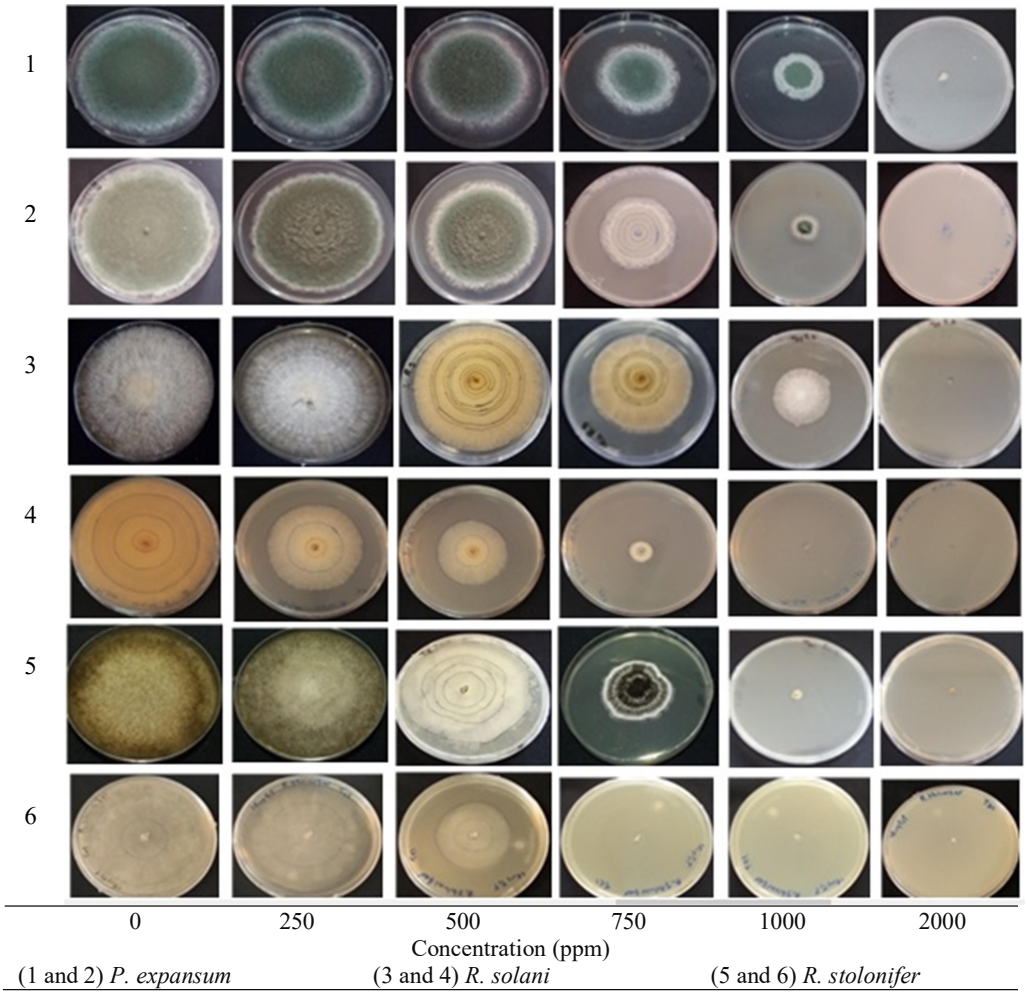

Figure 3. The antifungal activity of MPE, MPE-SLN, and SLN formulations without MPE on the mycelial growth of the tested phytopathogens. The colony diameter of fungi was measured daily of incubation at 25±3°C. 1, 3, and 5 rows) *Mentha×piperita* L. essential oil, 2, 4, and 6 rows) MPE-SLNs. Zero in this row represents the SLN formulations without MPE.

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Table 4. The results of the mean comparison of the effect of various concentrations (ppm) and various kinds of formulations(MPE, MPE-SLN, SLN formulations without MPE, and tween 80) on the inhibition percentage of the growth of the fungi pathogens. [a]

Formula-kind	Concentration (ppm)	*P. expansum*	*R. solani*	*R. stolonifer*
MPE	0	0 ± 0^{n}	0 ± 0^{n}	0 ± 0^{n}
	250	3.15 ± 0^{n}	0 ± 0^{n}	0 ± 0^{n}
	500	14.74 ± 0^{l}	2.11 ± 1.05^{n}	8.42 ± 0^{m}
	750	54.21 ± 1.39^{g}	11.4 ± 1.32^{ml}	49.47 ± 1.05^{h}
	1000	64.03 ± 1.32^{e}	51.05 ± 3.2^{gh}	100 ± 0^{a}
	2000	91.58 ± 0.52^{b}	100 ± 0^{a}	100 ± 0^{a}
MPE-SLNs	SLN without MPE	0 ± 0^{n}	0 ± 0^{n}	0 ± 0^{n}
	250	14 ± 0^{l}	39.33 ± 1.15^{i}	19 ± 1^{k}
	500	23.33 ± 3.21^{j}	60 ± 7^{f}	48.66 ± 3.21^{h}
	750	49.33 ± 5.03^{h}	83.33 ± 7.63^{c}	100 ± 0^{a}
	1000	77.66 ± 1.52^{d}	100 ± 0^{a}	100 ± 0^{a}
	2000	92.5 ± 0.5^{b}	100 ± 0^{a}	100 ± 0^{a}

[a] (Above columns indicate significant differences according to Duncan's multiple range tests at $P \leq 0.01$)

and its good lipid compatibility.

In colloidal systems, the key parameters affecting bioavailability and physical stability are particle size and size distribution (Piran *et al.*, 2017). The results indicated that the average particle size and PDI of the MPE-SLN formulations were rather stable and, respectively, less than 200 nm and 0.2, during 6 months of stability studies. These results were similar to those of previous findings (McDaniel *et al.*, 2019) that indicated the presence of essential oil did not affect the particle size of SLNs. The particle size of MPE-SLN was similar to that of the SLNs without MPE, the control (Table 3). In total, the particle size of SLNs was influenced by parameters such as synthesis method, compounds, and environmental conditions (time, temperature, pressure, the number of cycles, and equipment) (Nasseri *et al.*, 2016). A smaller particle size caused higher clarity of the formulations and, subsequently, reduced their sedimentation rate (Piran *et al.*, 2017). A PDI value of less than 0.2 indicated a narrow size distribution in accordance with previous studies (Montenegro *et al.*, 2017; Piran *et al.*, 2017). Another key parameter in the physical stability of the nanoparticles is the Zeta potential, which indicates the surface charge of the nanoparticles (Wu *et al.*, 2011). Based on our results, the Zeta potential of MPE-SLN formulations was negative and between -15 and -20mV. A Zeta potential value of approximately 20mV referred to acceptable stability in accordance with previous studies (WU *et al.*, 2008). The negative Zeta potential could be related to the components of the SLN formulation, such as the presence of the -COO- group in the used lipid, percirol, in the SLN formulations, which prevented the aggregation of particles by creating electrostatic repulsion (Fang *et al.*, 2008; Shi *et al.*, 2012; Zhao *et al.*, 2017). In addition, Tween 80 act as a hydrophilic non-ionic surfactant in these formulations, which increases its thermal stability, freeze thaw, and mechanical properties and also prevents aggregation through steric stabilization (Umoruddin *et al.*, 2019). The DSC diagrams of the SLN formulations indicated that the melting peak of the lipid cores of the SLN formulations (a temperature of 58˚C) was at a higher temperature than the bulk lipid melting point (a temperature of 56˚C). The melting peak of MPE was not observed in the DSC diagram of the MPE-SLN formulation, which could be due to the incorporation of essential oil into the lipid present in the formulation, that is, the synthesized formulation prevented the evaporation of the essential oil (Nasseri *et*

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al., 2016; Yang and Ciftci, 2016; Fazly Bazzaz *et al.*, 2018). The TEM image showed spherical and uniform particles with a size of about 100 nm, which was consistent with the data obtained from the particle size analysis (Figure 3).

In the present study, the antifungal activity of the MPE-SLN formulations and the free MPE was evaluated against fungal pathogens (Figure 4). Based on the results, an enhanced antifungal activity against fungal pathogens was observed when MPE-SLNs were used. MPE-SLN formulations had a significant difference from the results of the MPE on the inhibition growth of fungi pathogens (Table 3). It was also observed that none of the microorganisms were susceptible to SLN formulations without MPE, which indicated the effectiveness of essential oil enhanced in SLN formulations. The high antimicrobial activity of SLN formulations was dependent on the larger surface-to-volume ratio of particles, which potentially provided greater contact between the active substances and the microbial cell surface. On the other hand, the controlled release of the essential oil would promote the continuous diffusion of antimicrobials to the fungal membrane (São Pedro *et al.*, 2013; McDaniel *et al.*, 2019).

In accordance with the literature (Müller *et al.*, 2000; Katarija and Prajapati, 2013), the active ingredient of SLNs was protected by its solid matrix, ultimately protecting it from chemical degradation and improving the drug release profile. In this respect, volatility and evaporation of essential oils were decreased using SLN formulation, thus our results are consistent with Lai *et al.* (2006) and Nasseri *et al.* (2016).

The results of this study confirmed the findings of previous studies, which indicated that SLN formulation was a good carrier for incorporating the essential oils in good yield with acceptable physical stability, the reduction cytotoxicity, high effectiveness, and evaporation prevention of essential oil (Lai *et al.*, 2007; São Pedro *et al.*, 2013; Khameneh *et al.*, 2015; Nasseri *et al.*, 2016). Therefore, the nano-encapsulation of essential oils in drug delivery systems overcame the problems of using essential oils.

CONCLUSIONS

Indeed, this study points out that the antifungal activity of *Mentha×piperita* L. essential oil-loaded solid lipid nanoparticles was higher than that of the free essential oil. This was probably due to the high loading of essential oil in the formulation, the controlled release of essential oil, the high surface-to-volume ratio of the formulation, and increased contact of the formulation with the fungal cells. Therefore, this formulation is recommended to facilitate the use of essential oil and to utilize the properties of essential oil to preserve food and increase the shelf life of agricultural products after harvest. In order to commercialize this formulation on a large scale, it is necessary to investigate its effects on the organoleptic and sensory properties of the treated products.

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سنتز، بررسی خصوصیات و فعالیت ضد قارچی نانوذرات لیپیدی جامد حاوی اسانس نعناع فلفلی (*Mentha×piperita* L.) در شرایط درون شیشه‌ای

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چکیده

استفاده از اسانس‌ها و سیستم‌های دارورسانی جدید دو رویکرد برای کنترل قارچ‌های بیماری‌زا گیاهی در نظر گرفته شده است. این مطالعه با هدف سنتز، بررسی خصوصیات و فعالیت ضد قارچی نانوذرات لیپیدی جامد (SLNs)حاوی اسانس نعناع فلفلی (*Mentha×piperita* L. (MPE در مقایسه با فرم آزاد اسانس انجام شد. در این مطالعه، فرمولاسیون¬های SLNs حاوی MPE (MPE-SLNs) بوسیله هموژنیزاسیون با نیروی برشی بالا و تکنیک اولتراسوند سنتز شدند. سپس خواص فیزیکی، اندازه، شاخص پراکندگی، پتانسیل زتا، درصد انکپسولاسیون، تصاویر میکروسکوپ الکترونی و پایداری فرمولاسیون¬ها در طول مدت ۶ ماه نگهداری بررسی شدند. نتایج نشان داد که فرمولاسیون¬های MPE-SLNs دارای اندازه ذره¬ای ۴/۷ ± ۱۵۵/۵ نانومتر، شاخص پراکندگی ۰/۰۱۲ ± ۰/۱۵۶، پتانسیل زتای ۰/۸۷ ± ۱۵/۹۳- میلی¬ولت، درصد انکپسولاسیون حدود ۰/۸۸ ± ۸۸ درصد و خواص فیزیکی آن¬ها به مدت ۶ ماه پایدار بودند. همچنین نتایج نشان داد که حداقل غلظت بازدارندگی در شرایط درون شیشه¬ای برای فرم آزاد اسانس علیه میکروارگانیسم‌های قارچی Rhizoctonia solani و Rhizopus stolonifer به ترتیب ۲۰۰۰ و ۱۰۰۰ میکرولیتر بر لیتر و برای فرمولاسیون¬های MPE-SLNs به ترتیب ۱۰۰۰ و ۷۵۰ میکرولیتر بر لیتر بودند. بنابراین، فعالیت ضد قارچی فرمولاسیون¬های MPE-SLNs نسبت به فرم آزاد اسانس MPE اثر قابل توجهی داشتند. میکروارگانیسم¬های قارچی نسبت به فرمولاسیون¬های بدون اسانس حساس نبودند. با توجه به این نتایج می¬توان گستره¬ی وسیعی از غذاها و محصولات کشاورزی را بوسیله فرمولاسیون¬های MPE-SLNs بصورت سالم و ایمن نگهداری کرد.

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