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Antifungal Activity of Nanoemulsion of Iranian Tarragon (*Artemisia dracunculus* L.) Essential Oil

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HIGHLIGHTS

- *Artemisia dracunculus* (tarragon) Essential Oil (EO) showed significant antifungal potential.
- Main fragments of tarragon EO were beta-cis-ocimene, estragole, and beta-trans-ocimene.
- Growth inhibitory activity of tarragon EO enhanced when was encapsulated as nanoemulsion.

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Acronyms and abbreviations
EO=Essential Oil
GC/MS=Gas Chromatography Mass Spectroscopy
MFC=Minimum Fungicidal Concentration
MIC=Minimum Inhibitory Concentration

ABSTRACT

Background: Despite the considerable activity of herbal Essential Oils (EOs) as safe food preservatives, problems such as high volatility, low water solubility, and low stability in adverse environmental conditions restrict their use in food products. This work aimed to investigate *in vitro* antifungal activity of oil-in-water nanoemulsion of Iranian *Artemisia dracunculus* L. (tarragon) EO.

Methods: Nanoemulsion of tarragon EO was formed by ultrasound method through blending 10 wt% of tarragon EO, 85 wt% water, and the mixture of 5 wt% surfactants (Tween® 80/Span® 80). The droplet size and zeta potential were measured. The antifungal activity was evaluated against four different fungi, *Aspergillus niger*, *Penicillium* spp., *Fusarium* spp., and *Saccharomyces cerevisiae* through determining Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC), and mycelial growth test. Data were statistically analyzed by the software of SPSS 22.0.

Results: Main fragments of tarragon EO found to be beta-cis-ocimene, estragole, and beta-trans-ocimene. Nanodroplets had a zeta potential of -30 mV and an average diameter of 50 nm. For *A. niger*, *Penicillium* spp., *Fusarium* spp., and *S. cerevisiae*, the MIC and MFC values of nanoemulsion were identical and obtained at 1.50, 2.05, 1.61, and 1.14 µg/ml, respectively, while these values of free EO were higher and as follows: 2.81, 4.52, 3.75, and 2.40 µg/ml, respectively. Mycelial growth showed that encapsulated EO had the most fungitoxic potential against *A. niger* (inhibition 41%) and *S. cerevisiae* (inhibition 68%). Also, *Penicillium* spp. was the most resistant against both EO and nanoemulsion.

Conclusion: The growth inhibitory activity of tarragon was significantly enhanced when encapsulated as nanoemulsion.

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Introduction

Fungal contamination and spoilage of food products are considered as a major food industry and public health concern throughout the world. It has been a significant source of chronic human food-borne intoxications and

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also causes economic losses in different branches of the food industry (Aristil et al.,2020; Patriarca, 2016). So, antifungal chemical preservatives are being used to prevent the germination of fungal spores and extend the shelf life of the products (Lv et al., 2011). In recent years, due to increasing awareness of consumers about the harmful effects of synthetic food preservatives and demand for natural substitutes, the food industries and food science researchers search for nature-based compounds that show high preserving efficiency. In this regard, herbal extracts and Essential Oils (EOs) are the most popular compounds which exert considerable antimicrobial and antioxidant effects and most of them are being used as food flavours in different countries. Despite the considerable activity of herbal extracts and EOs as safe food preservatives, some problems restrict their use in food products. The most important problems are the high volatility of their phenolic compounds (which decrease the efficiency during the shelf life period), low solubility in water, and adverse effects of environmental conditions on their stability (Donsì et al., 2011). To protect the hydrophobic bioactive ingredients against harmful environmental condition and increase the solubility and stability, several coating techniques such as microencapsulation and nanoencapsulation in nature-based bioactive materials are invented.

Tarragon (tarkhoon in Persian) a genus with the scientific name of *Artemisia dracunculus* L. is originated from the Asteraceae family and a part of the herbaceous grasses (Obolskiy et al., 2011). It is used as a flavouring agent in Iranian dishes (yogurt, salads, marinades, soups, and barbecues) and also has many remedial properties in traditional medicine (Maham et al., 2014; Ventura-Martinez et al., 2020). The major ingredients of tarragon EO and extracts vary on basis of culturing region and harvesting season (Azizkhani et al., 2021; Lin and Harnly, 2012). Among the major ingredients in its EO, estragole, ocymene phellandrene, and limonene have the highest antimicrobial activity (Ayoughi et al., 2011). Kordali et al. (2005) evaluated the antifungal potential of the EOs of *A. dracunculus*, *A. absinthium*, *A. santonicum*, and *A. Artemisia spicigera*. The EOs expressed considerable antifungal activity against the growth of pathogenic fungi. As *A. dracunculus* is the most cultured and consumed species of *Artemisia* genus in Iran, it is worthy to study on improving its antimicrobial activity.

Antimicrobial EO nanoemulsions are prepared as oil droplets-in-water which are stabilized using surfactants (Donsì and Ferrari, 2016). They have activity against a wide range of fungi and spores. It is well demonstrated that the antimicrobial activity of EOs is strongly based on their molecular hydrophobicity, mainly their phenolic compounds which interact efficiently with the cell membrane lipids, so change the original cell wall molecular structure enhance the membrane permeability and cause the leak of ions and cytoplasmic content out (Moghimi et al., 2016). Therefore, encapsulation techniques such as nanoemulsifying can bring great benefits by improving I) the dispersibility of EOs in food matrices, II) physico-chemical stability of EOs, and III) the interaction of EOs with microbial cell walls (Donsì and Ferrari, 2016). Several studies have investigated the formulation and antifungal properties of EOs nanoemulsions (Moazeni et al., 2021; Pongsumpun et al., 2020; Wan et al., 2019). Balasubramani et al. (2018) formulated a nanoemulsion from EO of *Ocimum basilicum* L. leaves and reported that nanoemulsion exhibited significantly higher antibacterial activity against different pathogens, and also stronger antioxidant ability in comparison to non-encapsulated EO. Zhang et al. (2017) announced that blended cloves/cinnamon EO nanoemulsions have good structural, thermal, and storage stability compared with nonencapsulated EO, implying higher antimicrobial activity against the common microorganisms. These researches suggest potential application of nanoemulsions in food and beverage as they could help durable organoleptic properties and act as natural preservatives.

To the best of our knowledge, no work on the nanoemulsification of Iranian tarragon EO has been reported to evaluate its antifungal potential. Hence, the objective of this study was to formulate nanoemulsion of tarragon EO and investigate its characteristics and antifungal properties.

Materials and methods

Materials

Food grade Iranian tarragon EO was ordered from Barij Co. (Kashan, Iran). Tween® 80 (polyethylene sorbitan monooleate, 822187) synthetic grade and Span® 80 (sorbitan monooleate, 840123) synthetic grade was purchased from Merck-Millipore (Darmstadt, Germany). Purified water used in our experiments was obtained by filtration through 0.2 µm filters in a MilliQ system (Milipore Co., Bedford, MA, USA). For antifungal assays, Potato Dextrose Broth (PDB), Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Yeast Peptone Dextrose (YPD) broth, de Man, Rogosa and Sharpe (MRS) broth, and MRS agar were obtained from Sigma Aldrich (St. Louis, MO, USA).

EO analysis

Tarragon EO was analysed by Gas Chromatography (GC; Thermo Quest® 2000, UK). The chromatograph was equipped with a DB-5 capillary column (30 m×0.25 mm ID×0.25 µm film thickness; Agilent Technologies,

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USA). The following condition was used to acquire the data: initial temperature 50 °C; rate of increase of temperature 2.5 °C/min, final temperature 265 °C, and injector temperature 250 °C. Injection volume was 0.5 µl and employed applying the autosampler (autosampler 7,693-100 positions, Agilent Technologies, USA). Helium was used as the carrier gas and the split ratio was adjusted to 120. The column head pressure was recorded as 24.9 kPa. An Agilent Flame Ionization Detector (model 6890, Agilent Technologies Co., USA) run at 200 Hz was applied. Also, a Gas Chromatography/Mass Spectroscopy (GC/MS; Thermo Quest Finnigan®, UK) with the same capillary column and analytical conditions indicated above was used for EO analysis. The MS worked in the electron ionization mode with the ionization energy of 70 eV. Each component was identified on basis of comparing its relative retention time and mass spectra with that of standards (Guan et al., 2007). As reference points, N-alkanes (C8-C20) and the data reported in reference books and standard libraries (Wiley 275.L and Wiley 7n.L) (Adams, 2007) were used in the calculation of Relative Retention Indices (RRI).

Preparation of nanoemulsion

Oil in water emulsion (O/W) of tarragon EO was formed at ambient temperature by ultrasound method through blending 10 wt% of tarragon EO, 85 wt% water, and the mixture of 5 wt% surfactants (Tween® 80/Span® 80). Frequently, the method used for the selection of emulsifying agents (surfactants) is the Hydrophilic-Lipophilic Balance (HLB) technique. The HLB value of the mixed surfactant system was calculated through the equation below:

$$HLB=[(m_A\times HLB_A)+(m_B\times HLB_B)]/m_A+m_B \quad \text{[Equation 1]}$$

where m_A and m_B are the mass of surfactants A and B, respectively. HLB_A and HLB_B are considered the HLB value of surfactants A and B, respectively. In this study, Tween® 80 with a HLB value of 15.0 and Span® 80 with a HLB value of 4.3 were used for nanoemulsion preparation. The number HLB of 9 (Span® 80 and m_A: 56%; Tween 80 and m_B: 44%) was determined on basis of Equation 1 with the concentration of the mixed surfactants in the emulsion system (Rosen and Kunjappu, 2012).

Measurement of nanoemulsion droplet size

A Dynamic Light Scattering (DLS) technique was applied to determine the mean droplet size of tarragon EO nanoemulsion using a Malvern Zetasizer Nano Series (Nano ZS model ZEN 3600, Malvern, UK) apparatus operating at a fixed scattered angle of 173°. Measurements were carried out at 25±0.1 ºC in triplicate. The software used to collect and analyze the data was the Zetasizer Software (version 7.03).

Measurement of Zeta potential

The zeta potential of the nanoemulsion was measured in a disposable folded capillary cell model DTS1070 (Malvern Instruments, Worcestershire, UK) at 25 ºC applying the Zetasizer® Nano ZS (model ZEN 3600, Malvern Instruments, Worcestershire, UK). Before conducting measurements, cells were allowed to equilibrate at 25 ºC for 120 s (Shahavi et al., 2016). All experiments were repeated in triplicates.

Antifungal assays

Antifungal potential of EO and nanoemulsion was determined against *Saccharomyces cerevisiae* (PTSS 5177), *Aspergillus niger* (PTCC 5010), *Penicillium* spp., and *Fusarium* spp. through Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC), and mycelial growth tests according to Ribes et al. (2017).

A. niger, *Penicillium*, and *Fusarium* isolates were inoculated onto PDA plate and incubated at 25±1 °C for 7 days. *S. cerevisiae* was cultured in Yeast Peptone Dextrose (YPD) broth and incubated at 32±1 °C for 48 h. Then, the population of inoculums was adjusted to 10^6 Colony Forming Unit (CFU)/ml by applying a haemocytometer. EO and nanoemulsion were diluted in PDB media for molds and YPD broth for *S. cerevisiae* and transferred to a 96-well plate. The volume of PDB and YPD broth in each well was 180 µl and 20 µl of fungal inoculum was added to achieve a total volume of 200 µl. The final concentrations of EO and nanoemulsion were 0.2-10 µg/ml. The inoculated microplates were incubated at 25±1 °C (72 h) for molds and 32±1 °C (48 h) for the yeast. MIC was defined as the lowest concentration at which there is no visible growth. MFC was measured by the subculture of 50 µl from each well with no visible fungal growth on PDA (for molds) and Sabouraud Dextrose Agar (SDA, for the yeast) plates after incubating at 25±1 °C (Li et al., 2018).

To conduct mycelial/yeast growth inhibition experiment, 1 ml of EO or nanoemulsion was diluted with 9 ml PDA or SDA (45-50 °C) in a petri dish. Having cooled in room temperature, a PDA disc (with an approximate diameter of 5 mm) was taken from the edge of the fungal culture (7-day-old) and put on the center of the plates. The plates were incubated at 25±1 °C for 72 h and the growth diameter (mm) from the center to the edge of the petri dish was measured. The average growth measurement was carried out from three replicates of each fungal species. PDA/SDA plates treated with distilled water,

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without EO solutions, were used as a negative control. The equation of growth inhibition was calculated as:

Growth inhibition(%)=[($D_{control}$-D_{sample})/$D_{control}$]×100 [Equation 2]

where $D_{control}$ was the mean diameter (mm) of the fungal colony in control and D_{sample} was the mean diameter (mm) of the treated samples.

Statistical analysis

Each experiment was done in triplicate and all the data were statistically analyzed by the software of SPSS 22.0 (SPSS Inc., Chicago, IL, USA), using one-way analysis of variance and two-sample t-test. The significant differences were determined at the 95% level.

Results

EO components

GC/MS analysis expressed the presence of 34 components in tarragon EO. Main components of the EO include estragole (81.89%), beta-cis-ocimene (4.62%), beta-trans-ocimene (3.44%), l-limonene (1.67%), and eugenol methyl ether (1.49%). As seen, the main components of the EO in this study were composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents. The main component of tarragon was estragole (81.89%) that was isomer of anethole (Table 1).

Nanoemulsion droplet size and zeta potential

In the present work, according to DLS measurements nanoemulsion droplets were found to have a mean diameter of 50 nm. The tarragon nanoemulsion droplets had a negative zeta potential of -30 mV.

Antifungal activity

The MIC and MFC of free tarragon EO and its nanoemulsion were determined for each fungus (Table 2). For *A. niger*, *Penicillium* spp., *Fusarium* spp., and *S. cerevisiae*; the MIC and MFC values of nanoemulsion were identical and obtained 1.50, 2.05, 1.61, and 1.14 µg/ml, respectively, while these values of free EO were higher and as follows: 2.81, 4.52, 3.75, and 2.40 µg/ml, respectively. It was noticed that *S. cerevisiae*, as yeast, was more susceptible to the inhibitory effect of tarragon EO or nanoemulsion compared with *A. niger*, *Fusarium* spp., and *Penicillium* spp., as molds. The MICs and MFCs found for nanoemulsion were lower than those for free EO.

According to the results, the MIC and MFC values were decreased when the EO was encapsulated in nanoemulsion. As reported in Table 3 and Figure 1, mycelial growth assays revealed that free EO had weaker antifungal ability than nanoemulsion. The results of mycelial growth tests showed similar trend to MIC and MFC assays. Encapsulated EO had the most fungitoxic potential against *A. niger* (growth inhibition 41%) among molds and *S. cerevisiae* (growth inhibition 68%) (p<0.05); and *Penicillium* spp. was the most resistant mold against both free EO, and nanoemulsion with 3% and 13% mycelial growth inhibition, respectively (p<0.05).

Table 1: Chemical composition of *Artemisia dracunculus* L. Essential Oil (EO) identified by Gas Chromatography/Mass Spectroscopy (GC/MS)

Compounds	Amount (%)	Retention Index	Compounds	Amount (%)	Retention Index
1-R-α-Pinene	0.64	932	Eugenol methyl ether	1.49	1404
Camphene	0.05	943	Caryophyllene	0.1	1418
Sabinene	0.05	968	Decalactone	0.17	1467
β-Pinene	0.09	974	Germacrene D	0.21	1479
β-Myrcene	0.11	986	β-Ionone	0.09	1482
L-Limonene	1.67	1027	Bicyclogermacrene	0.16	1494
β-trans-Ocimene	3.44	1039	α-Farnesene	0.1	1504
β-cis-Ocimene	4.62	1050	β-Sesquiphellandrene	0.09	1521
α- Terpinolen	0.33	1085	(-)-Spathulenol	0.17	1578
Allo-Ocimene	0.71	1128	Caryophyllene oxide	0.14	1583
Estragol	81.89	1215	Spathulenol	0.91	1609
Pulegone	0.05	1240	Caryophyllenyl alcohol	0.05	1647
Bornyl acetate	0.19	1287	Herniarin	0.13	1717
Thymol	0.14	1290	2-Pentadecanone, 6,10,14-trimethyl-	0.06	1840
Carvacrol	0.31	1306	1,2,4-Triazolo[4,3-a]pyridine, 3-phenyl-	0.2	1933
δ-Elemene	0.09	1335	Phytol	0.48	2110
Eugenol	0.44	1358	Total	99.73	932
(E)-Methyl cinnamate	0.43	1380			

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Table 2: Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) of tarragon Essential Oil (EO) and its nanoemulsion against tested fungi

Fungi	MIC (µg/ml)		MFC (µg/ml)	
	Free EO	Nanoemulsion	Free EO	Nanoemulsion
Aspergillus niger	2.81 [a*]	1.50 [b]	2.81 [a]	1.50 [b]
Penicillium spp.	4.52 [a]	2.05 [b]	4.52 [a]	2.05 [b]
Fusarium spp.	3.75 [a]	1.61 [b]	3.75 [a]	1.61 [b]
Saccharomyces cerevisiae	2.40 [a]	1.14 [b]	2.40 [a]	1.14 [b]

[*] Different letters in the rows indicate statistically significant difference (p<0.05).

Table 3: Antifungal activity of tarragon Essential Oil (EO) and its nanoemulsion against tested fungi

Fungi	Growth inhibition zone (mm)	
	Free EO	Nanoemulsion
Aspergillus niger	3.3±0.09 [a*]	13.4±1.70 [b]
Penicillium spp.	1.5±0.11 [a]	6.5±0.10 [b]
Fusarium spp.	2.9±0.54 [a]	11.8±1.2 [b]
Saccharomyces cerevisiae	1.5±0.30 [a]	5.6±1.15 [b]

[*] Different letters in the rows indicate statistically significant difference (p<0.05).

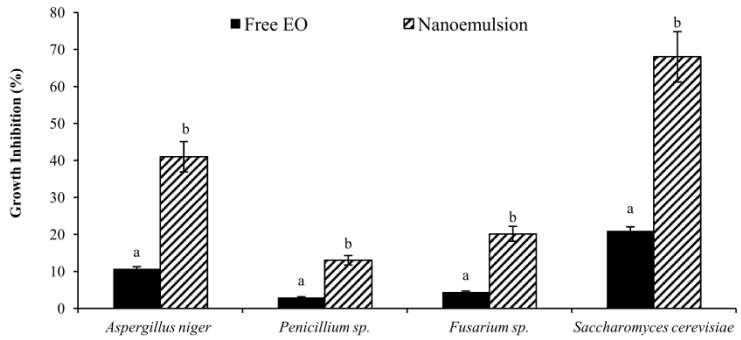


Figure 1: Inhibitory potential of tarragon Essential Oil (EO) and its nanoemulsion against growth of fungi

Discussion

According to the results of GC/MS analysis, the main fragments of tarragon EO were terpenes and terpenoids. Our results confirm the earlier report of Fraternale et al. (2015) that major volatile constituents obtained from the aerial parts of Italian *A. dracunculus* L. were estragole (73.3%), limonene (5.4%), (E)-β-ocimene (5.3%), β-pinene (3.4%), and (Z)-β-ocimene (3.0%). Also, Ayoughi et al. (2011) reported that the main components of *A. dracunculus* L. were anethole (51.72%), beta-ocimene (8.32%), methyl eugenol (8.06%), limonene (4.94%), and linalool (4.90%). In the above mentioned studies, the main components of tarragon were anethole isomers which are similar to our findings. Also, the amount of beta-ocimene isomers in our work is similar to the data of the above research. Other compounds like limonene and eugenol methyl ether were found lower than 2% in this

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work while other studies reported higher amount of limonene (Verma et al., 2010) and eugenol derivatives (Ayoughi et al., 2011; Fraternale et al., 2015). The dissimilarity between the amount of chemical compounds in tarragon EO originated from various regions is due to the difference in soil composition, climate, time of harvesting, method of drying the herbs, EO extraction process, etc. (Dhifi et al., 2016).

As it is announced by previous studies, particle size and distribution are determining factors in the physical and rheological properties of colloidal systems such as stability, the release of the core material and encapsulation efficiency (Hasani et al., 2015). In the present work, according to DLS measurements, nanoemulsion droplets were found to have a mean diameter of 50 nm that provides high emulsion stability. As described by Saifullah et al. (2016), the nanoemulsions possess droplet size <100 nm. The nanoemulsions, similar to the conventional emulsions, are thermodynamically defined as metastable since phase separation happens over time; but as no gravitational separation or particle aggregation occurs due to the weak attractive force between the droplets, nanoemulsions are kinetically stabile (McClements and Rao, 2011).

The zeta potential is a measure of the magnitude of the electrostatic or charge repulsion/attraction between particles which is a major factor in phenomena such as dispersion, flocculation, or aggregation, and hence a key parameter for evaluating the stability of dispersions, emulsions, and suspensions (Dickinson, 2009). In the present work the tarragon nanoemulsion droplets had a negative zeta potential (-30 mV). According to Shahavi et al. (2016), particles with zeta potential higher than +30 mV and lower than −30 mV show considerable stability; such high stability of nanoemulsions at high zeta potential value is due to the presence of high surface charge which decreases the risk of coagulation. This is because of electrostatic repulsion between particles bearing same electric charges. Also, they reported that nanoemulsion mean diameter under 50 nm with zeta potential below -30 mV implies on high stability of the particle. The results of this study indicated that tarragon nanoemulsion possessed relatively high stability, reasonable dispersion, and resistance against coagulation.

The MIC and MFC of free tarragon EO and its nanoemulsion were determined for each fungus. For *A. niger*, *Penicillium* spp., *Fusarium* spp., and *S. cerevisiae*; the MIC and MFC values of nanoemulsion were identical and obtained 1.50, 2.05, 1.61, and 1.14 µg/ml, respectively, while these values of free EO were higher. It was noticed that *S. cerevisiae*, as yeast, was more susceptible to the inhibitory effect of tarragon EO or nanoemulsion compared with *A. niger*, *Fusarium* spp. and *Penicillium* spp., as molds. The MICs and MFCs values for nanoemulsion were lower than those for free EO.

According to our data, nanoencapsulated EO showed the highest fungitoxic activity against the growth of *A. niger* with the inhibition potential of 41%. It significantly reduced the growth rate of *S. cerevisiae*. *Penicillium* spp. was the most resistant fungus upon treatment with free EO and nanoemulsion.

Studies on antifungal effects of EOs and related nanoemulsions of several species of various herbal plants have expressed that they have different degrees of growth inhibition activities. Donsì et al. (2011) announced that in their review study of nanoencapsulation of some EOs in order to improve their antimicrobial activity, the MIC values of the antimicrobial compounds encapsulated as nanoemulsions were lower or equal to free compounds, suggesting facilitation of EO transport through the cell wall of the target pathogens. Abd-Elsalam and Khokhlov (2015) in a study on antifungal activity of eugenol nanoemulsion against *F. oxysporum* announced more disruption of the fungal structures in mycelia and spores treated with nanoemulsion compared with free eugenol. The greatest antifungal activity of this EO was found against *F. oxysporum* DQ086833 (inhibition zone of 5 cm at 2% EO concentration) followed by *F. oxysporum* AY264267 (inhibition zone of of 4.5 cm). The nanoemulsion at concentration of 5% completely inhibited the growth of all *F. oxysporum* isolates. The same results were obtained in the present study about the antifungal effect of EO nanoemulsion. Several works have also evaluated the inhibitory effect of EOs and nanoemulsions on yeasts, among which *S. cerevisiae* is the most investigated. According to their results, yeast cells showed lower MIC for encapsulated compounds (Donsì et al., 2012; Zhang et al., 2014). Bedoya-Serna et al. (2018) investigated the antifungal effect of oregano (*Origanum vulgare*) EO nanoemulsion and reported that upon treatment with EO and nanoemulsion the highest occurrence was expressed by *Penicillium* spp. followed by *Fusarium* spp. For *Fusarium* sp. and *Penicillium* sp., the MIC values were 0.2 µg/ml and 0.3 µg/ml, respectively. In their study, comparing the inhibitory activity of the free oregano EO with nanoemulsion showed that the encapsulation of the EO improved its antifungal effect against *Fusarium* spp., while for *Penicillium* spp., the use of encapsulated oregano EO decreased its inhibitory activity against this mold. In the present work encapsulation of tarragon EO resulted in enhanced antifungal activity against both *Fusarium* spp. and *Penicillium* spp.

On the other hand, several researches have reported results in contrast to ours, e.g., in a study by Li et al. (2018) both MIC and mycelial growth results showed that finger citron (*Citrus medica* L. var. *sarcodactylis*)

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nanoemulsion had weaker fungitoxic activities compared with free EO against *A. niger*. The MIC and the mycelial growth tests expressed that nanoemulsions exerted weaker antifungal effects compared to free EO. In MIC assays, nanoemulsions inhibited the growth of *A. niger* and *P. citrinum* at concentrations of 20 and 30 µl/ml, similar to free EO. In this regard, Ribes et al. (2017) reported that bergamot EO nanoemulsion had a weaker inhibitory effect than free EO on mycelial growth of *A. niger*. Also, in another work D-limonene nanoemulsions exhibited weaker fungicidal activity against *S. cerevisiae* than unencapsulated D-limonene (Zhang et al., 2014). It should be noted that nanoencapsulation may exert positive or negative effects on the antimicrobial efficacy of EOs and not necessarily enhances the antimicrobial activity of all EOs. It seems that macromolecules such as emulsifiers reduce the antifungal activity and also some components in the nanoemulsions act as nutritive substrates for the microorganisms (Donsì et al., 2012; Donsì and Ferrari, 2016).

Several works have reported the antifungal activity of tarragon EO, showing different ranges of growth inhibition (Céspedes et al., 2006; Kordali et al., 2005) and our study demonstrated that nanoencapsulation of this EO improved its antifungal activity compared with free EO. According to the previous studies the inhibitory potential of EOs depends on parameters such as species taxonomy, region of culturing, and climate, agronomic conditions, EO extraction technique, variety, and amount of the active compounds in the EO and microbial genus (Falcone et al., 2005). In regard with nanoemulsified EOs, type of emulsion, chemical properties of the emulsifier, formulation of the emulsion, the average size, and the surface charge of nanoemulsion droplets can affect the antimicrobial potential of the EOs (Donsì and Ferrari, 2016). It seems that molecular structure of emulsifiers can affect antifungal potential of nanoemulsions and some macrostructures cause emulsions exert a weak microbial inhibitory effect. Also, ingredients used in designing nanoemulsion might act as nutritive substances and improve fungal growth (Donsì et al., 2012).

Conclusion

On the basis of the results reported in the present work and previous papers, it can be concluded that the EOs consisting of high amount of phenolic compounds and oxygenated monoterpenes possess relatively stronger antifungal activity. Compared with the free tarragon EO, this work also indicated that the growth inhibitory activity of tarragon was significantly enhanced when encapsulated as nanoemulsion. These results suggest designing of EO based nanoemulsions as efficient antifungal additives in the food stuffs and also conducting organoleptic assays in order to obtain optimum nanoemulsion formula.

Author contributions

M.A. and F.T. designed the study; F.J.K. conducted the experimental work; F.T. analyzed the data; M.A. wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

There is no conflict of interest in the study.

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