Eurasian Journal of Food Science and Technology 2021; Vol:5, Issue:2, pp:105-116

Effects of Electrospinning on Antifungal Properties of Thyme and Cardamom Oils

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Abstract

Thyme and cardamom oils are known to be various health benefits along with their antimicrobial properties are used in foods as additives or condiments. Such functional properties of these essential oils (EOs) stem from bioactive and volatile components that are very sensitive to ambient conditions. Encapsulation of EOs provides protection of core material. Electrospinning is an easy, effective and relatively low-cost encapsulation method. There is no clear statement on how electrospinning affect the functional properties of active material that is to be encapsulated. The aim of this study was to investigate the effects of uniaxial and coaxial electrospinning on antifungal effects of thyme and cardamom oils, which had proven their antifungal effects, against isolates of *Aspergillus carbonarius* 35-03X1 and 39-04X4. The uniaxial and coaxial electrospinning encapsulated thyme oil exhibited less antifungal effect against 39-04X4 compared to non-encapsulated thyme oil. No antifungal effect of encapsulated cardamom oil was detected. Electrospinning inhibited the antifungal effect of cardamom oil. The outcomes of this study can help effect of electrospinning on functional properties of active materials.

Keywords: Thyme oil, cardamom oil, antifungal, electrospinning, encapsulation, *Aspergillus carbonarius*

Research article
Received date: 10 May 2021
Accepted date: 9 September 2021

INTRODUCTION

Essential oils (EOs) composed of various bioactive agents are strong, bitter and volatile. These oils are obtained by distillation from seeds and tissues of plants (Singh et al., 2002). Most of EOs are generally considered as GRAS (Generally Recognized as Safe) by the Food and Drug Administration (FDA) due to fact that they are not phytotoxic or harmful for environment (Da Cruz Cabral et al., 2013; Holley and Patel, 2005). EOs have antimicrobial effects on microorganisms because of their aromatic contents; they can be used as sedative and analgesic as well (Bakkali et al., 2008). These oils have antibacterial and antifungal phytochemical ingredients such as thymol, eugenol, menthol, carvacrol, benzoic acids, phenolic acids and flavone (Souza et al., 2005). The antimicrobial activity of EOs is affected by various parameters. For instance, high oil concentrations have generally more antimicrobial activity. The solubility and the diffusion ability of EOs should be taken into account as well. In addition, antimicrobial effect is stronger as exposure time to EOs increases (Seow et al., 2014).

In other words, antimicrobial activity of EOs depends on type of oil and microorganism (Friedman et al., 2004), concentration of EOs, exposure time, pH, temperature and other ambient conditions (Seow et al., 2014). Some species of black *Aspergillus* species produce mycotoxins like ochratoxin (Abarca et al., 2003; Abarca et al., 2004; Cabaňes et al., 2002) and fumonisin (Frisvad et al., 2007; Frisvad et al., 2011, Perrone et al., 2011). *Aspergillus carbonarius, Aspergillus niger, Aspergillus acidus, Aspergillus brasiliensis and Aspergillus ibericus* are common species of black *Aspergillus* (Nielsen et al., 2009). *A. carbonarius* can be isolated from several foods such as grapes, coffee, spices, cereal and cereal products (Joosten et al., 2001; Kapetanaku et al., 2011; Nakajima et al., 1997). Most of *A. carbonarius* isolates are highly ochratoxigenic. At tropical or semi-tropical areas, *A. carbonarius* is source of ochratoxin A (Joosten et al., 2001; Nakajima et al., 1997; Patino et al., 2005; Pitt, 2000). The maximum growth rate of *A. carbonarius* is observed at a_w 0.95 between 0.8-0.95 and 30°C between 15-35˚C at a study. Results showed that *A. carbonarius* cannot grow at below 0.85 a_w (Romero et al., 2007). Ochratoxin A is produced at 0.86-0.94 a_w values by *A. carbonarius* (Esteban et al., 2006). Some environmental factors such as temperature, pH and aw affect fungal growth and ochratoxin synthesis (Gürhayta and Çağrındı, 2016). EOs and their terpenoid components can easily dissolve in lipids. Generally, EOs damage the biological membrane and microbial membrane-catalyzed activities like, e.g. respiratory pathway due to their lipophilic properties. Therefore, they can act as antimicrobial and antifungal agent by their components (Knobloch et al., 1989). There are various studies on the antifungal affects of EOs. The summary of some of the studies were given at Table 1. EOs should be protected from ambient conditions through processing and storage for exhibiting their desirable effects. Encapsulation can isolate and immobilize sensible bioactive components, therefore the substance is protected from the surrounding environmental conditions. Simultaneously, the controlled release and stabilization of bioactive materials can be provided by the matrix. In addition, core material can be added to foods without affecting taste, aroma and texture of food (Augustin and Hemar, 2009).

Table 1. Various studies on the antifungal affects of EOs.

Essential Oil	Mold	Concentration	Medium	Effect	MIC or MFC*	Reference
Thyme (*Thymus serpyllum* L)	*A. carbonarius*		Potato dextrose agar (PDA)	Antifungal effect	1.25 µl/ mL	Sokolic-Mihalak et al. (2012)
Boldo, poleo, clove, anise and thyme	*A. niger aggregate and A. carbonarius*	500 µL/L	Peanut meal extract agar	*A. carbonarius* inhibition: 52.8 %		Passone et al. (2012)
Cuminum cyminum, *Ziziphora clinopodioides* and *Nigella sativa*	*A. parasiticus*				*C. cyminum*: MIC: 1.6 mg/mL MFC: 3.5 mg/mL	Khosravi et al. (2011)
Marjoram	*A. niger*, *A. carbonarius* and *A. wentii*	2.5 mL/100 mL	Czapek yeast autolysate agar (CYA)	*A. carbonarius* inhibition: 95.6 %		Kocić-Tanackov et al. (2012)
Cinnamon, clove, anise, cardamom	*A. flavus*	10 mg/mL		Cardamom and anise: no antifungal effect, Cinnamon and cloves: 100% inhibition	MIC of cinnamon: 4 mg/mL, MIC of clove: 2 mg/mL	AikoMehta (2013)

Basil (*Ocimum basilicum* L.)	*Alternaria spp., A.flavus, Botrytis cinerea, Cladosporium herbarum, Eurotium amstelodami* and *E. Chevalieri*			*E. chevalieri* most sensitive, *A. flavus* most durable		Jakowienko et al. (2011)
Lippia rugosa, Plectranthus glandulosus, Clausena anisata and *Vepris heterophylla*					*Aspergillus spp*.: *L. rogusa*: 0.5 mg/mL, *P.glandulosus*:1.5 mg/mL *Fusarium* spp.: *L. rogusa*: 0.3 mg/mL, *P.glandulosus*:1.2 mg/mL *Penicilium* spp.: L. rogusa: 0.6 mg/mL, *P.glandulosus*:2.0 mg/mL	Aoudou et al. (2010)
Origanum vulgare L. (Lamiaceae)	*Aspergillus, Penicillium* and *Fusarium spp.*	80 and 40 µl/mL		Prevention of spore formation	80- 20 µl/mL	Carmo et al. (2008)
Thymus mastichina L. ssp. mastichina	*Aspergillus species (A. flavus, A. parasiticus, A. terreus, A. ochraceus, A. fumigatus ve A. niger)*				MIC: 1500-2100 µg/mL MFC: 2.0-2.4 mg/mL	Fraternale et al. (2003)
Thyme, cinnamon, marigold, spearmint, basil and quyssum	*Fusarium species*	500-3000 ppm	Potato dextrose agar (PDA)	Thyme: fungistatic: 250 ppm, fungicidal: 500 ppm Basil: fungistatic: 2000 ppm, fungicidal: 3000 ppm		Soliman and Badeaa (2002)
Marjoram (*Origanum vulgare*)	*A. flavus, A. parasiticus, A. ochraceus* and *F.moniliforme*	1000 ppm	Yeast extractsucrose (YES) broth	antifungal		Basílico and Basílico (1999)
Cardamom	*A. ochraceus*	0.05 %		Prevention of *A. parasiticus* growth		Badei (1992)

*MIC: minimum inhibition concentration; MFC: minimum fungisidal concentratio

When capsule size is less than 100 nm, capsules are named as nanocapsules (Augustin and Hemar, 2009). The nanoencapsulation is an encapsulation technique that uses nanotechnology processes such as nanoemulsification and nanostructuration. Nanoencapsulation provides final product functionality including controlled release of the core which is expected to be maintained during storage (Quintanilla-Carvajal et al., 2010). Nanocapsules present several potential advantages such as better encapsulation efficiency, protection, distribution for the bioactive ingredients compared to microcapsules. Furthermore, the improved bioavailability or overcoming incompability can be handled by nanocapsules (Xiao et al., 2013).

Donsi et al. (2011) nanoencapsulated terpenes and D-limonene and tested on *Escherichia coli*, *Lactobacillus delbrueckii* and *Saccharomyces cerevisiae*. Nanoencapsulation of terpenes decreased the minimum inhibition concentration (MIC) and minimum bactericial concentration (MBC) values or they were equal to the values of the unencapsulated mixture, although the nanoencapsulation of D-limonene reduced just the MIC. Low concentrations of the nanoencapsulated terpenes retarded the microbial growth (1.0 g/l terpenes) or inactivated the microorganisms (5.0 g/l terpenes) by adding to them into the fruit juices. In addition, they caused minimal organoleptic changes in fruit juices (Donsì et al., 2011). In an another study, thymol and carvacrol oils were encapsulated by the zein nanoparticles using the liquid-liquid dispersion method. After that, the nanoparticules were tested against *E. coli*. 0.8-1.8 log CFU/ml decrease was reported (Wu et al., 2012). Zhaveh et al. (2015) encapsulated *Cuminum cyminum* by chitosan (CS)–caffeic acid (CA) nanogel and tested it against *Aspergillus flavus*. The MICs of encapsulated and non-encapsulated *C. cyminum* were 350 and 650 ppm against *A. flavus*, respectively (Zhaveh et al., 2015). In a similar study, thyme was encapsulated by using CS and benzoic acid (BA) nanogel to improve its antifungal properties and half-life. Under sealed condition, the MIC of the CS-BA encapsulated EO was 300 mg/l, although the free thyme extract prevented the growth of *A. flavus* at 400 mg/l. Under non-sealed condition, higher concentration of encapsulated thyme oil (500 mg/l) was needed to fungi inhibition and free oils could not provide the complete inhibition, even at concentrations as high as 1000 mg/l. It was found that the nanogel encapsulation significantly increased half-life and the anti-fungal properties of thyme essential oil (Khalili et al., 2015).

Electrospinning is an encapsulation method, which can produce fibers with a diameter of 2 nm to few micrometers obtained uniaxially or coaxially. Electrospinning process is performed at room conditions, i.e. ambient temperature and pressure, which may be more suitable for encapsulation of sensitive bioactive components. In uniaxial electrospinning one feed solution, containing wall polymer and active material is present, whereas at coaxial electrospinning, there are two immiscible co-flowing fluids that are fed by two different capillary tubes. One of them carries inner fluid whereas the other one delivers the shell fluid. At the end of capillary tubes, electrical charge is applied on co-fluids and coaxial jet moves to the collector (Díaz et al., 2008; Bhardwaj and Kundu, 2010).

Wen et al. (2016) produced electrospun polyvinyl alcohol/cinnamon essential oil/β-cyclodextrin (PVA/CEO/b-CD) and applied on *Staphylococcus aureus* and *E. coli*. The MIC and MBC values were approximately 0.9-1 mg/mL and 7-8 mg/mL, which is a strong antimicrobial activity against the *S. aureus* and *E. coli* (Wen et al., 2016).

Using high voltage, i.e. over 10 kV, the electrospinning may have some undesirable effects on the active material that is to be encapsulated. Or, during electrospinning process, active materials which are sensitive to ambient conditions such as light, temperature, humidity may have been affected inversely or lost their functions. Eventhough, electrospinning encapsulation produce high yields in terms of efficiency, this point should be investigated. In this study, we used thyme and cardamom oil, which was proven that they have antifungal effect, to investigate the influence of electrospinning on their antifungal properties. Therefore, the aim of this study was to investigate the effects of electrospinning encapsulation of thyme and cardamom oils on their antifungal properties against *Aspergillus carbonarius* 35-03X1 and 39-04X4 isolated from dried figs from Aegean Region.

MATERIALS AND METHODS

Materials

At this study, *A. carbonarius* 35-03X1 and 39-04X4 which were isolated from dried figs from Aegean Region, were used to determine antifungal activity (Karbancıoğlu-Güler, 2008). Thyme (0.864 g/mL) and cardamom (0.899 g/mL) oils were supplied from IFF Inc., (International Flavors and Fragrances Inc., Kocaeli, Turkey) and were stored in original packages at 4ºC until further analysis. Gelatin and acetic acid were purchased from Sigma-Aldrich, USA.

Encapsulation of EOs

All details of the electrospinning and the concentration of feed solutions were given in elsewhere (Arıkan et al., 2016). Electrospun mats were cut as 1x1 cm or 2 x 2 cm. Electrospun samples of uniaxial (U) and coaxial (C) encapsulated thyme (T) and cardamom (C) oils were named as UT-1, UT-2, CT-1, CT-2, UC-1 and UC-2 based on their dimensions in the petri dishes (code 1 is for 1x1 cm and code 2 is for 2x2 cm), respectively. The gelatin electrospun mats without EOs were used as control.

Preparing mold isolates and spore suspensions

To prepare mold isolates and spore suspensions, the methods given in (Yavuz, 2015) were applied. In this study, *Aspergillus carbonarius* 39-04X4 and 35-03X1 isolates were used. Spore concentration of moulds were adjusted $1x10^6$ spore/mL in distilled water with 0.05% Tween 80.

Antifungal effects of electrospun EOs

The antifungal effects of electrospun mats were determined by using disc diffusion method against *A. carbonarius* (Maruzzella and Liguori, 1958). 100 µL spore suspension was inoculated onto MEA by spreading plate method. Electrospun mats were sterilized under UV irradiation for 2 h (each side for 1 h). Sterilized electrospun mats were placed on the inoculated media. After incubation at 30 °C for 48 h, the antimicrobial properties of the mats were assessed by measuring the inhibition zone diameter (including nanofiber mat) in each inoculated plate. All analysis were carried out in triplicate for each mat.

RESULTS and DISCUSSION

Electrospinning of EOs

Thyme oil encapsulated by uniaxial and coaxial electrospinning whereas cardamom oil can only be encapsulated by uniaxial electrospinning (Arıkan et al., 2016). The uniaxial electrospun nanofibers of gelatin without EOs were also obtained as a control sample.

Antifungal properties of electrospun EOs

The uniaxially nanofiber encapsulated cardamom oil (UC-1 and UC-2) exhibited no antifungal effect on *A. carbonarius* isolates. This may be because of the morphology of the electrospun samples with cardamom oil, which were not smooth and continuous as a fiber form (Arıkan et al., 2016). Probably, the volatile active components of cardamom oil evaporated during electrospinning. In this case, then, for volatile component of cardamom oil should be solubilized in the feed solution (the solvent was a mixture of acetic acid:water) by addition of a proper material or a surfactant for the electrospinning to obtain full and homogenous solution with all volatiles are present. Another point is that, Yavuz (2014) reported that the antifungal effect of cardamom oil was low compared to thyme oil against *A. carbonarius* 3904-X4.

The petri dishes, which include uniaxial and coaxial nanofibers of thyme oil, and uniaxial nanofibers of gelatin against 39-04X4 were given at Figure 1, 2 and 3, respectively. As it can be clearly seen from Fig 1 and Fig 2, uniaxially (UT-1 and UT-2) and coaxially (CT-1 and CT-2) nanofiber encapsulated thyme oil had an antifungal effect compared to the control, because there were clear and distinct inhibition zones in the petri dishes. The inhibition zones for UT-1 and UT-2 were 1.1-0.9 mm and 2.5-2.9 mm, respectively. This antifungal effect was more profound for the uniaxially encapsulated samples, probably due to that thyme oil in these samples was readily to show its effect. On the other hand, the inhibition zones were smaller for the CT-1 (1.0-0.8 mm) and CT-2 (1.2-0.9 mm) compared to the inhibition zones of UT-1 and UT-2. This may be attributed to that thyme oil encapsulated in the coaxial geometry of the nanofiber system. Therefore, the exhibiting its effect compared to uniaxial geometry was probably extended.

Yavuz (2015) reported that non-encapsulated thyme oil at 1:10 dilution had the inhibition zone of 16 mm against 39-04X4. It appears that uniaxial electrospinning of thyme oil (2.5 and 2.9 mm for UT-2) decreased the inhibition zone almost 6.4 and 5.5 times compared to the inhibition zone of non-encapsulated thyme oil. This was that even the ratio of thyme oil to gelation solution in the uniaxial electrospinning feed solution was 1:9 (Arıkan et al., 2016) whereas thyme oil was diluted 1:10 given by Yavuz (2015). This decrease was probably due to that possible matrix effect of encapsulation system or the high applied voltage during electrospinning. However, it may be concluded that electrospinning decreased the antifungal effect of thyme oil.

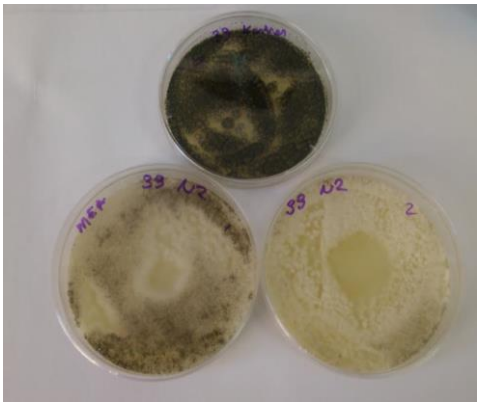

Figure 1. The petri dishes of control (up) and UT-1 (uniaxial electrospun thyme oil, 1 cm x1 cm) (on the left) and UT-2 (uniaxial electrospun thyme oil, 2 cm x 2 cm) (on the right) against 39-04X4

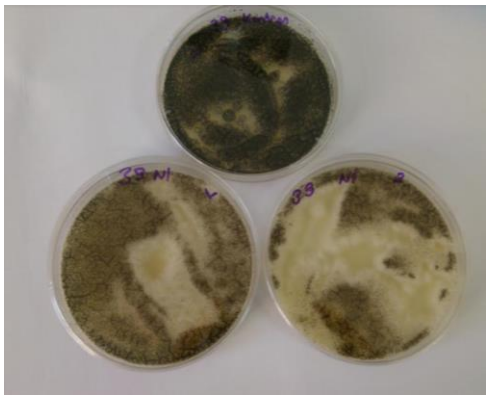

Figure 2. The petri dishes of control (up) and CT-1 (coaxial electrospun thyme oil, 1 cm x1 cm) (on the left) and CT-2 (coaxial electrospun thyme oil, 2 cm x 2 cm) (on the right) against 39-04X4.

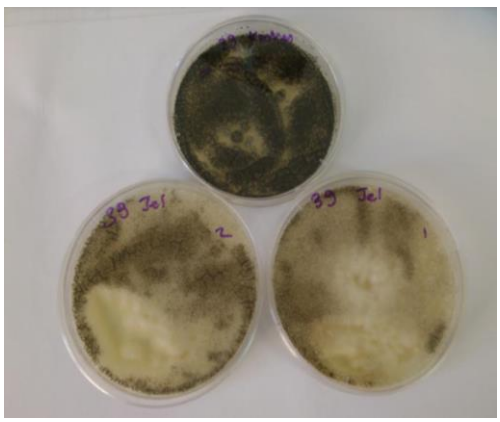

Figure 3. The petri dishes of control (up) and uniaxial electrospun gelatin with 2 cm x2 cm (on the left) and uniaxial electrospun gelatin with 1 cm x 1 cm (on the right) against 39-04X4

The petri dishes, which include uniaxial/coaxial nanofibers of thyme oil and uniaxial nanofibers of gelatin against 35-03X1 were given at Figure 4, 5 and 6, respectively.

As it can be clearly seen from Fig 4 and Fig 5, uniaxially (UT-1-X1 and UT-2-X1) and coaxially (CT-1-X1 and CT-2-X1) nanofiber encapsulated thyme oil had an antifungal effect compared to the control, because there were clear and distinct inhibition zones in the petri dishes. The inhibition zones for UT-1-X1 and UT-2-X1 were smaller than 2.0 mm and 2.5-2.4 mm, respectively. This antifungal effect was more profound for the uniaxially encapsulated samples, probably due to that thyme oil in these samples was readily to show its effect. On the other hand, the inhibition zones were smaller for the CT-1-X1 (1.0-1.0 mm) and CT-2-X1 (1.4-2.0 mm) compared to the inhibition zones of UT-1-X1 and UT-2-X1. This may be attributed to that thyme oil encapsulated in the coaxial geometry of the nanofiber system. Compared to the effect on 39-04X4, coaxial nanofibers with thyme oil had more effect on 35-03X1.

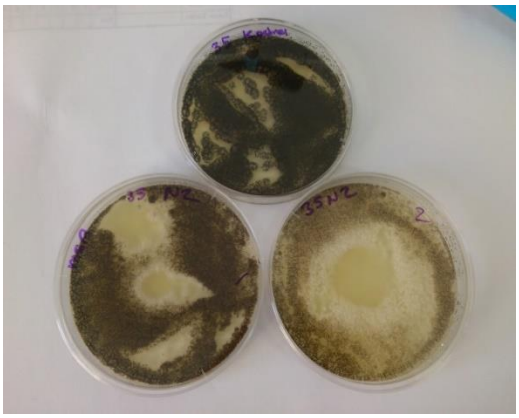

Figure 4. The petri dishes of control (up) and UT-1-X1 (uniaxial electrospun thyme oil, 1 cm x1 cm) (on the left) and UT-2-X1 (uniaxial electrospun thyme oil, 2 cm x 2 cm) (on the right) against 35-03X1

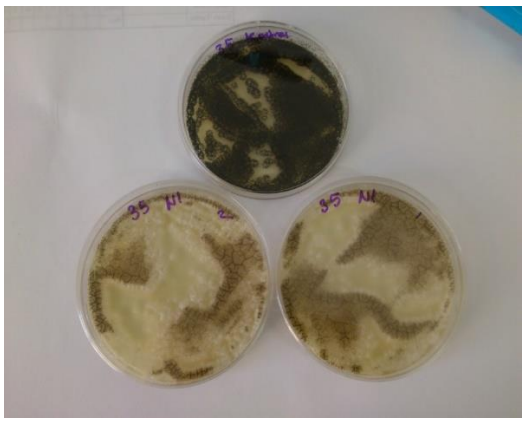

Figure 5. The petri dishes of control (up) and CT-1-X1 (coaxial electrospun thyme oil, 1 cm x1 cm) (on the left) and CT-2-X2 (coaxial electrospun thyme oil, 2 cm x 2 cm) (on the right) against 35-03X1

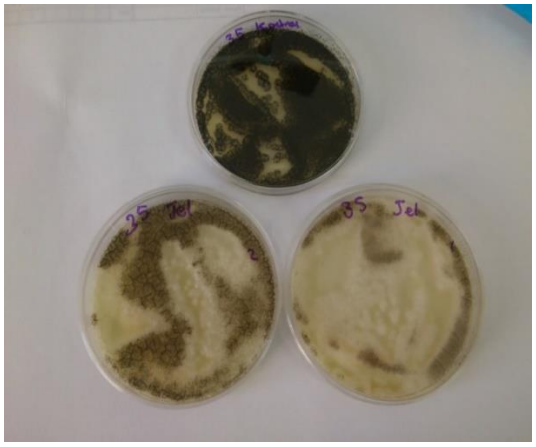

Figure 6. The petri dishes of control (up) and uniaxial electrospun gelatin with 2 cm x2 cm (on the left) and uniaxial electrospun gelatin with 1 cm x 1 cm (on the right) against 35-03X1

In order to evaluate the antifungal property of encapsulated thyme oil; the same protocol was applied to gelatin nanofibers, which had no antifungal effect itself (Fig 3 and Fig 6). As seen from Fig 3 and Fig 6, there was no distinct inhibition zone in the petri dishes for gelatin nanofibers. However, it can be seen a delay for spore growth, meaning that gelatin may contain peptide part or parts, which may have antifungal effect at the nanoscale to take effect in the petri dishes. Actually, the petri dishes contained uniaxial and coaxial nanofiber encapsulated thyme oil exhibited inhibition zone with the delay for spore growth as well. This may be due to gelatin nanofiber, which was used as wall material in the encapsulation system.

CONCLUSIONS

The outcomes of this study suggested that uniaxially nanofiber encapsulated thyme oil can be used as an antifungal. On the other hand, coaxially encapsulated thyme oil has an extended antifungal effect due to the geometry of the coaxial electrospinning encapsulation system. The electrospinning decreased the antifungal effect of uniaxial and coaxial encapsulated thyme oil. The nanofiber encapsulation of cardamom oil should be investigated in detail, because it has an antifungal effect without encapsulation. It is interesting to study the effect of electrospinning on antimicrobial properties of essential oils which may help to understand the encapsulation mechanism during electrospinning and antimicrobial effect of encapsulated essential oils. Furthermore, their release from the encapsulation system can be tailored as needed.

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