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Novel aspect of essential oils with a history of antimicrobial activity to be used as a potential source of carbon and nitrogen for the nutrition of *Candida* pathogens

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ABSTRACT. Essential oils (EOs) are complex aromatic compounds with a broad range of biological activities. Those with a history of antimicrobial action were evaluated in this study as a nutritional source for pathogenic *Candida*. Clinical isolates of *Candida* spp. were cultivated on two types of media with six types of EOs (aniseed, purple nutsedge, harmal, camphor, black seed, and linseed). One medium contained only glucose (GM) and the second contained only peptone (PM). Many EOs in GM encouraged the growth of several *Candida* isolates. The EOs in PM showed low support to many isolates. Isolate 2 of *C. albicans* was the most effective strain to use with nearly every EO tested in the two media. In conclusion, EOs can be used as a source of carbon and nitrogen depending on the type of EO and fungal species. In an environment with less nutrients, EO may be recommended as a nutrient source for fungi rather than for its known antifungal activity.

Keywords: aniseed; black seed; essential oil; Candida; linseed.

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Introduction

Essential oils (EOs) are a mixture of low molecular weight constituents with aromatic characters (Morsy, 2017; Moghaddam & Mehdizadeh, 2017; Fokou, Dongmo,& Boyom, 2020). They contain more than 300 diverse compounds (Dhifi, Bellili, Jazi, Bahloul,& Mnif, 2016) that determine the specific aroma, flavor and fragrance of the plant that contains it (Moghaddam & Mehdizadeh, 2017). Most important characteristics of EOs are an odorous, complex composition, typically liquid though a few are solid or semisolid at room temperature and soluble in alcohol and ether, but not in water (Morsy, 2017; Moghaddam & Mehdizadeh, 2017). They are produced as secondary metabolites in part or whole plants and serve the plant to control the invasion of other organisms (Fokou et al., 2020). Humans have used EOs for many purposes, including food and the production of cosmetics, perfumes and pharmaceutical drugs (Dhifi et al., 2016; Moghaddam & Mehdizadeh, 2017). EOs also have many other biological activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer, allelopathy and cytotoxicity (Goyal, Sharma, Upadhyay, Gill, & Sihag, 2014; Fatma, Fatiha, EI-Attafia, & Noureddine, 2016; Bajpay, Nainwal, Singh, & Tewari, 2018; Ashfaq, Khan, & Ali, 2021).

Around 60 plant families have EOs (Moghaddam & Mehdizadeh, 2017). EOs belong to numerous chemical groups including alcohols, ethers, ketones, aldehydes, amines, esters, phenols and hydrocarbons (Dhifi et al., 2016; Moghaddam & Mehdizadeh, 2017). The main chemical components of EO are terpenes or their derivatives oxygenated terpenoids (80%) and phenols in the form of phenylpropanoids (Dhifi et al., 2016; Morsy, 2017; Moghaddam & Mehdizadeh, 2017; Fokou et al., 2020). Terpenes are synthesized in the plant via the mevalonate pathway, while phenols are synthesized via the shikimate pathway (Dhifi et al., 2016). The odor of EO is mainly related to the phenylpropanoid derivatives (Moghaddam & Mehdizadeh, 2017; Fokou et al., 2020).

The correlation between EOs and fungi is mainly highlighted in numerous studies based on the antifungal action of EOs against various species of fungi (Shojaii & Fard, 2012; Sun, Shahrajabian, & Cheng, 2019; Bajpay et al., 2018; Ashfaq et al., 2021).The nutritional value of EOs for fungi is obscure and not clear, and no studies mention it. There are few studies on plant oils contaminated by fungi which may be considered as an indicator of the ability of fungi to use an EO as a nutrient source. An investigation of contaminated fungi in six vegetable oils found 16 yeast and 35 mold species (Okpokwasili & Molokwu, 1996). Approximately 73 microorganisms,

including fungi, were isolated from contaminated soils containing different types of vegetable oils (Popoola & Onilude, 2017). Chakrabarti (1987) mentioned that many fungal species have been reported to infect seed oil from time to time in India of seeds under field or storage conditions.

The possibility of uses EO that has a history of antimicrobial activity as a carbon or nitrogen source for *Candida* species was evaluated in the present study.

Material and methods

Essential oils

Six types of essential oils (EOs) were selected for consideration due to their potential antimicrobial activities (Shojaii & Fard, 2012; Bajpay et al., 2018; Sun et al., 2019; Ashfaq et al., 2021). The EO with a purity of more than 90% of aniseed (*Pimpinella anisum* L.; family Umbelliferae), purple nutsedge (*Cyperus rotundus*; family Cyperaceae), harmal seeds (*Peganum harmala* L.; family Zygophyllaceae), camphor (*Cinnamomum camphora*; family Lauraceae), black seeds (*Nigella sativa* L.; family Ranunculaceae), and linseeds or flax (*Linum usitatissimum* L.; family Linaceae) were purchased from Hemeni-Karachi, Pakistan.

Fungal isolates

Ten species of *Candida* were isolated from patients with vulvovaginal candidiasis attending private gynecological clinics in Karbala city in February 2021. Vaginal swabs were collected and cultured on Sabouraud's Dextrose agar (HiMedia, India). Inoculated plates were incubated at 37°C for 24-48 hours. The identification of the fungi was initially based on the morphological characteristics of the yeasts examined microscopically. The diagnosis of species was confirmed by the Vitek® 2 system (BioMérieux, France) using Vitek® 2 YST ID diagnosis cards for yeast.

Culture media

Two types of media were prepared to test the suitability of each EO as a nutritional source for *Candida* species. The first was peptone medium (PM) composed of peptone 10 g, agar 15 g and 1 L of distilled water with sugar depletion as a source of carbon. The second was glucose medium (GM) composed of glucose 20 g, agar 15 g and 1 L distilled water with peptone depletion as a source of nitrogen. Media were sterilized by autoclaving at 121°C with 15 psi for 15 min. The EO was mixed with melting media at a concentration of 10 µL EO mL^{-1} of medium and poured into a sterilized plate. All experiments were performed in triplicate.

Fungal growth

Inoculum of isolated yeast was prepared using yeast culture in a sterile test tube with Sabouraud's dextrose broth (HiMedia, India) and incubated at 35°C for 24 hours. The turbidity of the growth suspension was adjusted to the 0.5 McFarland standard with sterilized physiological normal saline to approximately 1×10^8 cfu mL^{-1} 100 μL^{-1} of the standard fungal count was inoculated on a plate of prepared media by spreading with sterilized cotton swab. The inoculated plate was incubated at 35°C for 24 hours. Media free of EO was used as a control. The degree of growth was determined on the basis of a model plus symbol (+) where the increase in the number of plus symbols indicated increased in growth matter (Bajpay et al., 2018).

Results

Five *C. albicans* isolates, four *C. glabrata* isolates and one *C. utilis* isolate were diagnosed from the clinical specimens. The culture of *Candida* spp. on six types of EO showed variable degrees of growth. Harmal and camphor EOs in GM revealed more encouraging growth of all *C. albicans* isolates, two of *C. glabrata* and one of *C. utilis* (Figure 1). Good growth was also observed on GM with the EO of black seeds, while few *C. albicans* and *C. utilis* showed growth on media containing EOs of purple nutsedge and linseed. There was no role for aniseed EO to promote the growth of isolates (Table 1).

Species of *Candida* also showed variable ability to grow on GM with different EOs. *C. albicans*-2 was found to have very good growth capacity on five of the six EOs, followed by *C. albicans*-1 and *C. albicans*-5 (Figure 1). Meanwhile, two *C. glabrata* isolates were found to be unable to grow on any of the EOs tested as opposed to *C. utilis* that developed to a very high degree on all EOs tested. Negative results were achieved when isolates were cultured on an EO-free control medium of GM (Table 1) (Figure 1-A).

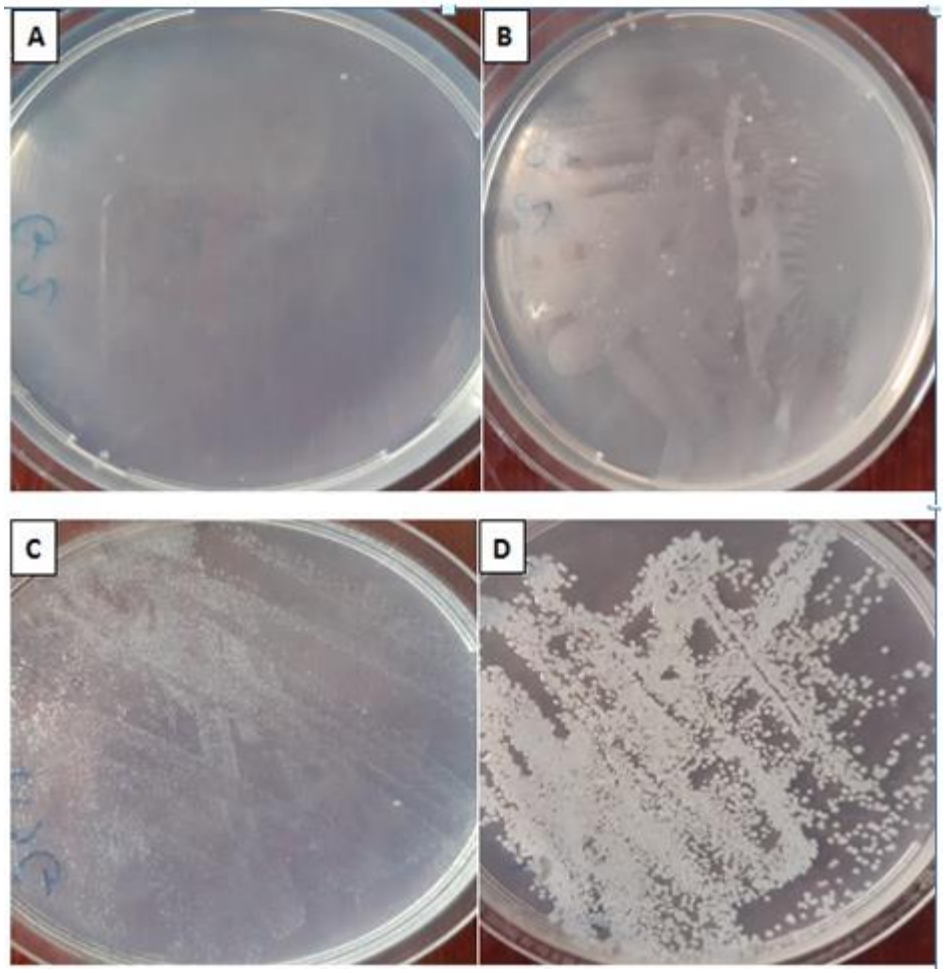

Figure 1. *Candida* spp. growing on media with essential oils. A: Negative growth of *C. albicans* on control medium with glucose only (GM). B: *C. albicans* growing on control medium with peptone only (PM) (++). C: *C. albicans*-2 growing on GM with harmal essential oil (++).D: *C. albicans*-2 growing on PM with harmal essential oil (++++).

Table 1. *Candida* spp. growing on glucose medium (GM) with essential oils (EOs).

Candida isolate	Essential oil						Control
	Aniseed	Purple nutsedge	Linseed	Harmal	Black seed	Camphor	
C. albicans-1	-	-	+	++	+	++	-
C. albicans-2	-	++	++	++	++	+++	-
C. albicans-3	-	-	-	++	++	++	-
C. albicans-4	-	-	-	+	-	++	-
C. albicans-5	-	+	++	++	-	++	-
C. glabrata-1	-	-	-	+	+	++	-
C. glabrata-2	-	-	-	-	-	-	-
C. glabrata-3	-	-	-	-	-	-	-
C. glabrata-4	-	-	-	+	+	+	-
C. utilis	-	+	+	+	+	+	-

+: positive growth and an increase in the number of pluses indicating increased growth rate. -: Negative growth.

The growth of *Candida* isolates on PM with EOs showed different results than on GM. Growth of all isolates was observed on PM with all types of EO, but was not different from that observed on the control medium (Figure 1-B). This means that EOs did not support the growth of a large number of *Candida* species. Although aniseed EO showed no effect on the growth of isolates on GM, its mixture with the PM support growth of one *C. albicans* isolate (*C. albicans*-1) and two of *C. glabrata* (*C. glabrata*-1 and *C. glabrata*-4). The EO of linseed in PM also assisted the growth of two *C. albicans* and one *C. glabrata* isolates. Other EO types were observed to improve growth of only one *Candida* isolate, with the exception of camphor EO (Table 2).

At the species level, *C. albicans*-2 was exactly like the ones on GM that developed very well on PM with almost all types of EO (Figure 1). Meanwhile, it was found that *C. glabrata* isolates had difficulty developing on many EOs. This was also observed with *C. utilis* which did not manage to grow on all EOs except with

camphor EO. However, low growth rate was observed in many isolates on PM with certain EO types, especially for nutsedge EO as compared to the control (Table 2).

Table 2. *Candida* spp. growing on peptone media (PM) with essential oils (EOs).

Candida isolate	Essential oil						Control
	Aniseed	Purple nutsedge	Linseed	Harmal	Black seed	Camphor	
C. albicans-1	++++	+	++	++	++	+++	++
C. albicans-2	++	+++	++++	++++	+++	++++	++
C. albicans-3	++	+	++	++	++	++	++
C. albicans-4	++	+	++	++	++	++	++
C. albicans-5	++	+	+++	++	++	++	++
C. glabrata-1	++++	+	++	++	++	++	++
C. glabrata-2	++	++	++++	++	++	++	++
C. glabrata-3	++	++	++	++	++	++	++
C. glabrata-4	+++	+	++	++	++	++	++
C. utilis	++	++	+	++	+	+++	++

+: positive growth and an increase in the number of pluses indicating increased growth rate. -: Negative growth.

Discussion

Fungi need a wide range of elements to survive and continue to grow. Carbon and nitrogen are the most essential components for growing fungi. Yeasts can use different sources of nitrogen, but they are mainly dependent on glucose as the main source of carbon (Kingsbury, Goldstein, & McCusker, 2006).

The EOs selected for the current study are proven by numerous studies to have antifungal action against different fungal species (Shojaii & Fard, 2012; Bajpay et al., 2018; Sun et al., 2019; Ashfaq et al., 2021). From another perspective, a structural review of these EOs found that they contain a lot of carbon and nitrogen. This view may further the purpose of this study in terms of the potential use of these EOs as a nutrient source for *Candida* when there is insufficient glucose or peptone. The current results confirm this proposal in which many isolates of *Candida* revealed good growth on media with different EOs. *Candida* spp. and other yeasts showed in a previous study that they have growth ability on palm and corn oils (Okpokwasili & Molokwu, 1996). Previous results also demonstrated that linseed EO can support the growth of two species of *Aspergillus* (*A. fumigatus* and *A. niger*), *Melarmpsora lini* and *Fusarium oxysporum* f.sp. *lini* (Mondal & Nandi, 1984; Chakrabarti, 1987). In addition, several species of fungi, including *Candida* spp., have been successfully grown on various types of EO and make a change in these EO, such as the reduction of oil moisture (Okpokwasili & Molokwu, 1996). It has been demonstrated that *C. parapsilosis* can survive in olive oil for more than 18 months which can be increased to more than 24 months in dilute oil (Ciafardini, Cioccia, & Zullo, 2013). However, fungi can use EO as food through lipolytic activity of their enzyme (Chakrabarti, 1987). This lipolytic degradation is the primary reason that *C. parapsilosis* and *C. valida* have used palm oil as a source of nutrients (Popoola & Onilude, 2017). Lipase is the major lipolytic enzyme that gives *Aspergillus fumigatus* and *A. niger* the ability to break down linseed EO as a nutrient (Mondal & Nandi, 1984).

The EOs in the current study showed no growth of many *Candida* isolates in the absence of glucose when cultured in medium with peptone only (PM). In fact, EO compositions are enriched with carbon atoms, but they bind in a complex framework with very large quantitative and qualitative variability (Dhifi et al., 2016; Morsy, 2017; Moghaddam & Mehdizadeh, 2017; Fokou et al., 2020). Most of the carbon in the EO is found in double bonds that make the EO unsaturated (Fokou et al., 2020). Generally, glucose and fructose are the primary source of carbon for the growth of *C. albicans* (Lok et al., 2021). In the absence of such types of sugar, fungi may get carbon from other available sources. Despite the structural complexity of carbon-containing compounds in EO, fungi may use EO as a source of carbon for fatty acid metabolism which may explain the reduction in the amount of carbons in vegetable oil contaminated with fungi (Chakrabarti, 1987). There are four major groups of EO components with high carbon content; terpenes, terpenoids, non-terpenoids and phenylpropanoids (Morsy, 2017). Phenylpropanoids that occur as phenols or phenol ethers are the most enriched groups of EO with carbon (Moghaddam & Mehdizadeh, 2017). These types of components are already identified within the EOs of the present study. Black seed EO contains more than 32 complex compounds with high carbon content (Ashfaq et al., 2021). These compounds are mainly represented by thymoquinone as an essential component, terpenoid, and non-terpenoid types, and linoleic and oleic acids as fatty acids (Ghahramanloo et al., 2017). Analysis of the EO of purple nutsedge rhizomes revealed a high content of

carbon-rich compounds such as α-cyperone, myrtenol, caryophyllene oxide, oxo-α-ylangene, α- and β-pinene, and trans-pinocarveol (Bajpay et al., 2018). Although linseed is low in carbohydrates, its EO contains many types of carbon-rich compounds such as omega-3 and omega-6, fatty acids (α-linolenic acid, oleic, linoleic, palmitic and stearic acids) and lignans (Goyal et al., 2014). However, the complexity of the EO compositions and the absence of free sugar make it difficult for *Candida* spp. to get carbon from these compounds.

The results of this study showed that camphor and harmal EOs support the growth of many *Candida* isolates on GM. Camphor EO contains several compounds containing carbon without nitrogen constituents. Terpenes are an important component of camphor EO that is found in higher amounts with varying amounts of fatty acid (3-methyl-2-butenoic acid) and octanoic acid (Zhang et al., 2023). Such terpenes are structurally composed of volatile hydrocarbons with a general formula $(C_5H_8)_n$ (Yadav, Yadav, & Goyal, 2014). Most of the terpenes of camphor EO are D-camphor, 1,8-cineole, α-pinene, β-pinene, α-caryophyllene, and linalool (Pragadheesh, Saroj, Yadav, & Chanotiya, 2013; Guo et al., 2016). Although glucose may be used by *Candida* species on GM that was used in this study, camphor EO with its terpenes content increases the growth of many of these fungi. This unexpected result may be difficult to explain, particularly the fact that terpenes have shown a strong antifungal effect rather than a nutritional role (Zhang et al., 2020). Fries (1973) found such a result after studying the effect of many terpenes (longifolene, α-pinene, β-pinene, Δ-3-carene, and limonene) on the growth of 20 species of wood-decomposing fungi. Four species demonstrated an improvement in growth after being exposed to a gaseous form of terpenes. Interestingly, the mycelial growth of one species, *Coniophora puteana*, that exposure to longifolene increased two-fold after the increased concentration of glucose in the medium. A possible explanation of this fungal growth in a medium containing terpenes and glucose can be related to the nature of terpenes. Terpenes have low molecular weight and water insoluble characteristics that make it difficult to dissolve in the medium (Yadav et al., 2014). Having high levels of glucose in contact with terpenes may increase their weight (Wu & Maravelias, 2018). Thus, the presence of terpenes in the glucose-containing environment, as in the GM media in this study, can contribute to increased fungal growth.

Harmal EO, on the other hand, contains both a large amount of carbon source in the form of glycosides and nitrogen in the form of alkaloids (harmine, harmaline, harmalol and harmol) with a variable amount of tannins, saponins, terpenoids and steroids (Apostolico et al., 2016; Fatma et al., 2016). Thus, the presence of harmal EO in glucose-containing media may be a source of carbon and nitrogen to *Candida* growth.

The absence of peptone in the medium with glucose-only (GM) indicated a negligible effect of peptone on the growth of many isolates of *Candida* in the present study. In general, peptone can be used by many species of fungi as a primary source of nitrogen compared with ammonium and other nitrogen sources as with *Scleroderma sinnamariense* (Bechem, 2012). Cruz, Cilli, and Ernandes (2002) found that the presence of peptone as a nitrogen source in glucose-containing media influenced the metabolic rate of yeast and increased its biomass while maintaining its sustainability. The biomass of *Candida utilis* NOY1 cultured on tuber waste was increased after the addition of peptone (Ouedraogo et al., 2017). Generally, nitrogen-containing compounds in the EOs may take the form of methyl anthranilate, indole, pyridine and pyrazine (Morsy, 2017). Although nitrogenous compounds occur in a few types of EO, the amount of these compounds varies from one EO to another (Morsy, 2017; Moghaddam & Mehdizadeh, 2017). Propanoids of black seed EO contain nitrogen in their structural complexes with carbon atoms (Ashfaq et al., 2021). Harmal alkaloids contain a high amount of nitrogen which could provide a good source of nitrogen for different fungi (Fatma et al., 2016). Additionally, many amino acids within a protein structure can be used by fungi as a source of nitrogen (Bechem, 2012). Such a protein is found in large amount in the linseed EO (Goyal et al., 2014).

Aniseed EO supported weak growth of *Candida* isolates in the medium with glucose only (GM), while few isolates developed well on the medium with peptone only (PM). These findings may suggest that the aniseed EO content of carbon has no effect on *Candida* spp. growth, as opposed to nitrogen content. Aniseed EO contains a large amount of carbon and nitrogen which can make it a good source for these elements. The most identifiable compositions with a large amount of carbon in the aniseed EO are the anethole, coumarins, fatty acids such as palmitic and oleic acids, and carbohydrate, while the nitrogenous compounds are represented by proteins (18%) (Shojaii & Fard, 2012; Sun et al., 2019).

The results of the present study showed that the ability of *Candida* species to grow on various types of EO varied. This degree of variability also occurred at the strain level. The number one isolate of *C. albicans* (*C. albicans*-1) showed a high growth rate in both media with different types of EO. One of the 23 *C. parapsilosis* strains (QU110) revealed a strong ability to degrade different EOs by active lipase production compared to

other strains (Ribas et al., 2019). *C. albicans* isolate CaLIP10 was one isolates of *C. albicans* also reported to be able to use different kinds of oils without specifying any (Lan et al., 2011). However, lipase is an active enzyme for degraded oils and over 50% of the yeasts can produce different types of lipase enzymes with a molecular weight between 33 and 65 kD (Vakhlu & Kour, 2006). The type of lipase and its enzymatic activity may vary depending upon the species of *Candida* and the growing conditions (Ribas et al., 2019). Nine genes coding for lipase (LIP2-LIP10) were identified in *C. albicans* that encoded high flexible active lipases working on various type of oils (Hube et al., 2000). Thus, *C. albicans* may produce extra and intracellular lipase once they have been grown in different media (Jatta, Gunasekaran, & Mohan, 2009). The source of carbon and nitrogen can influence the production and activity of lipase from *Candida* species (Ribas et al., 2019), while the length of carbon in fatty acids has no such influence (Lan et al., 2011). Other factors related to culture conditions also affected the activity of the lipase of *Candida* spp., such as incubation time, temperature, pH and medium compositions (Jatta et al., 2009).

The antimicrobial effects of selected EOs in this study have been demonstrated in numerous studies of the growth of a microorganism on a standard medium that is enriched with suitable amounts of carbon and nitrogen sources. The interesting point can be ruled out from the current results is that the absence of essential nutrients such as carbon or nitrogen can convert the EO from toxic material into a good source of nutrients. Bioremediation is a close term which can be used to explain this behavior. Many species of fungi, including *Candida* spp., may use toxic substances as a source of nutrients in the absence of a natural supply of essential elements. Toxic engine oil is broken down with an efficiency of 95.42% by *Candida tropicalis* and *Aspergillus clavatus* and transformed into nutritional materials (Mbachu, Chukwura, & Mbachu, 2018). Five species of yeasts were found to be highly effective in using eight types of heavy metals and toxic compounds in wastewater as a source of nutrients to increase their biomass and this increase may be more than 11 times in the presence of certain types of toxic metals like Pb^{2+} and Cd^{2+} (Nicula et al., 2023). Toxic crude petroleum is degraded by four species of *Candida* and converted into less complex compounds that have an advantage for fungal growth (AL-Otibi, AL-Zahrani, & Marraiki, 2022).

Conclusion

Essential oils can be used as a nutrient source to grow many species of *Candida*. Some EOs play the role of an improvement factor, in particular the harmal and camphor EO, to increase the growth of some *Candida* spp. rather than inhibit it in the presence of glucose. Thus, these EOs can be considered as an appropriate additional source of carbon that can be used to increase fungal growth. The EOs may also be regarded as a good source of nitrogen for *Candida* spp., depending on the type of EO. Species or strains may have an effect on the degradation ability of *Candida* to use an EO as a carbon or nitrogen source. One important thing about EOs with antimicrobial activity is that they can be used as a source of nutrients for fungal growth primarily under condition of deficiency in essential elements.

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