Controlling of four phytopathogenic *Aspergillus* species by plant essential oil

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ABSTRACT: The antifungal effect of different concentrations of juniper and ginger oil (0.5, 1, 2.5, 5, 7.5 and 10%) was tested against *Aspergillus niger, A.ochraceus, A. fumigatus and A. flavus* on the medium of potato dextrose agar, the diameter of the *Aspergillus* colony decreased with an increase concentration of juniper oil where the mycelial growth diameter was recorded 32, 29, 22 and 10 mm for *Aspergillus niger* at concentration 0.5 , 1, 2.5 and 5%, respectively comapared to control, but this decrease is very weak and when we used a concentration of 7.5% and above from juniper oil that prevented the growth of pathogenic fungi, on the other hand, the effect of ginger oil on the development and prevention of pathogenic fungi was 100% at 5, 7.5 and 10%. Therefore, the results of this study showed that the growth of fungi decreased significantly when using ginger oil, and therefore its effect was better than that of juniper oil.

Key words : *Zingiber officinale* - *Juniperus sp.* – *Aspergillus* - Essential Oil

1- INTODUCTION

The Essential Oil (EO) are acquired from all plant parts, for the most part from herbs and flavors [Fengfeng *et al.* 2017 and Ravindran *et al.* 2016]. The essential oil as an item made by mechanical processing, for example, distillation with either water or steam of natural materials (Nazzaro *et al.* 2017). Daferera *et al*., 2000; Sridhar *et al*., 2003 revealed the essential oil and their constituents have been found effective as the antifungal agent.

Significant constituents of these oils are phenolic compounds like thymol carvacrol or eugenol of which antimicrobial activity is especially recorded. There are non-phenolic essential oils such as some *Juniperus species* (Newall *et al.* 1996). They identified α-pinene, sabinene, β-pinene, β-myrcene, α-phellandrene, limonene and bornyl acetate as the major constituents of Junipers oil (Váczi *et al*. 2018). Be that as it may, the phenol derivatives, for example, gingerols is significant constituents of ginger oil (Wohlmuth *et al.* 2005) .The main predominant compounds in ginger oil were geranial, α-zingiberene, γ-eudesmol and *cis*-sabinene hydrate (Ramadan *et al.* 2014).. Malek *et al*. 2005 discovered high content of monoterpenoids in ginger volatile oil.

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Aspergillus is one of the most critical components of the fungal flora of stored food. The most frequent species involved in food pollution include *A. flavus, A. niger, A. fumigatus* and *A. parasiticus.* The more dangerous mycotoxins noted for their mutagenic and carcinogenic effects include aflatoxin and ochratoxin produced respectively by *A. flavus* and *A. niger*. *Aspergillus ochraceus* is mainly responsible for Ochratoxin A "OTA" contamination in grains during storage (Hua *et al.* 2014 and Sokoli *et al.* 2012). Therefore, The used essential oils to inhibit some growth pathogenic fungi, It is a potential alternative to the use of chemical pesticides, which have had implications for human health and the environment (Xing *et al*. 2014). It is well established that certain plants and their derivatives possess antimicrobial properties (Nguefack *et al*., 2004; Jazet *et al*., 2008).

The aim of this investigation was to examine the antifungal activity of juniper and ginger oil and against phytopathogenic fungi *Aspergillus niger, A.ochraceus, A.fumigatus* and *A.flavus*.

2- MATERIALS AND METHODS

2.1 - Fungal Strain

The *Aspergillus* species isolates were given from Department of Botany, Faculty of Science, Omar Al Mukhtar University, Libya. Tested strain was incubated at 28 °C in Petri dishes (9 cm diameter) on PDA (Potato Dextrose Agar) for 5–7 days and kept in refrigerator at 4^{o}C for further testing.

2.2- Extraction of essential oils from juniper and ginger

Three kilograms of each ginger rhizome (*Zingiber officinale*) and juniper leaves (*Juniperus sp.*) were gathered and its size was diminished with grinder. Then they were blended separately in water until overflow in the flask. Extraction of the essential oil was finished utilizing Clevenger Apparatus by the hydrogen extraction method. After extraction the oils were collected and put away in refrigerator at 4ºC until use. (Sefu *et al.* 2015)

2.3- Assessment of antifungal potentiality

The antifungal effect of juniper oil on the development of fungus, various concentrations of essential oils diluted with dimethyl sulphoxide (DMSO) 1:1 proportion was added into Potato dextrose agar (PDA) media in 0.5, 1 , 2.5 , 5 , 7.5 , 10 % concentration. After that the treated media (20 ml) was poured into a Petri dish and allowed to solidify. Then the fungal discs of 5 mm diameter from the young cultures were placed in the middle of petri dishes. Plates with just media and no oil were used as control. The experiments were done in triplicates. The petri dishes were covered with a parafilm and left to incubate at 28 °C for 7-10 days. Colony

diameters of tested fungi with essential oils were estimated and inhibition percentage of mycelial growth (MGI) by using the formula (Doğu & Zobar 2014).

MGI (%) = [(dc – dt) / dc] x 100

dc: mycelial growth diameter in control

dt: mycelia growth diameter in treatment

2.4- Statistical Analysis:

The data presented in this investigation were means of three replications, and the standard deviation (± SD) was found by the Past programs.

3- RESULTS AND DISCUSSION

Essential oils play an important role as a biocontrol in reducing fungal contamination and may provide a renewable source of beneficial fungicides. The main components of these essential oils are terpenes, carbohydrates, phenols, alcohols, ethers, aldehydes and ketones which accounts for the biological activity and fragrance. The discoveries of previous investigations of natural substance had revealed the inhibitory job of essential oils on various fungal and bacterial species (Rasooli *et al*. 2006 and Xing *et al*. 2014 and Yamamoto-Ribeiro *et al.* 2013). EO may have different effect on fungal species due to its effect on biosynthesis and organelles rather than just the cell wall. Some previous studies have demonstrated that natural and synthetic antifungal agents can cause a considerable diminishing in the quantity of ergosterol, which is the major sterol component in the fungal cell membrane (Tian *et al.* 2012).

The effect of antifungal activity of various juniper oil concentrations (0.5 , 1 , 2.5 , 5 , 7.5 , 10 %) on development of *Aspergillus* species. Results of table (1) exhibit that greatest growth values were recorded at the lower oil concentration (0.5%) for the tested fungi. The colony diameter of *Aspergillus* species gradually decreases as the concentration of juniper oil increases. Whereas, there is no growth at high concentrations of oil (7.5 and 10%). In this investigation, the use of juniper oil at a high concentration avoids the growth of pathogens *Aspergillus* species. In any case, the results showed the capability of *J. oxycedrus* ssp. oil as an antifungal agent, having a marked fungicidal effect. This outcomes concurrence with the results of previous investigations on the antimicrobial activity of *Juniperus* oil. In the studies of Hammer *et al.* 1999, the oil of *J. communis* demonstrated that there is no antimicrobial activity or was weak. The inability of juniper oil to inhibit *aspergillus* growth can be attributed to the absence of phenolic compounds as appeared in the results Cavaleiro *et al*. 2006.

As for ginger oil, this resulted in inhibition of the growth of the fungi tested in this study in oil dosages of 5, 7.5 and 10%, and recorded a 100% inhibition rate as shown in Figures 1, 2, 3 and 4 and there studies conducted on ginger oil and showed that it has Antimicrobial effects (Preedy, 2016). Suksrikarm B. (1987) reported that cinnamon oil and ginger oil separately inhibit numerous different microorganisms, including *Alternaria sp., Aspergillus sp., Canninghamella sp., Fusarium sp., Mucor sp.,* and *Penicillium sp*. Similarly, El-Fadaly *et al.* 2018 exhibited that Ginger essential oil showed high anti-fungal effects on the mycelia growth of *Alternaria panax, Botrytis cinerea, Cylindrocarpon destructans, Fusarium oxysporum, Sclerotinia sclerotiorum*, and *Sclerotinia nivalis*. Ginger essential oil showed high anti-fungal effects on the mycelia development of *Colletotrichum gloeosporioides* even at lower concentrations (Sefu *et al.* 2015). Sasidharan & Menon 2010 have revealed that the fresh oil of ginger was effective inducing the antimicrobial effects on *Aspergillus niger, Candida* and less effective against *Saccharomyces cerevisiae*. The previous studies Xing *et al.* 2014 and Yamamoto-Ribeiro *et al.* 2013 found that Fusarium verticillioides cells shrank and were clearly missing of cytoplasmic substance after treatment with ginger oil and cinnamon oil, respectively.

Table 1 : Effect of different concentration of juniper and ginger oil on the growth of *Aspergillus* species

Oil conc. (%)	*A.nigar*		*A.ochraceus*		*A.fumigatus*		*A.flavus*	
	Juniper oil	Ginger oil	Juniper oil	Ginger oil	Juniper oil	Ginger oil	Juniper oil	Ginger oil
Control	34 ±1.56	34 ±1.34	32 ±1.08	32 ±0.52	32 ±0.23	32 ±1.66	33 ±0.22	33 ±1.67
0.5	32 ±1.23	30 ±0.66	30 ±0.12	27 ±1.51	29 ±1.82	28 ±1.38	30 ±1.34	29 ±0.05
1	29 ±0.96	21 ±1.20	25 ±0.06	19 ±1.09	25 ±0.06	20 ±0.32	27 ±0.56	22 ±1.91
2.5	22 ±1.21	14 ±0.26	20 ±1.26	13 ±0.83	19 ±1.72	11 ±1.33	21 ±1.78	10 ±1.03
5	10 ±1.44	0	14 ±0.21	0	12 ±0.37	0	10 ±0.08	0
7.5	0	0	5	0	7±0.33	0	4 ±1.76	0
10	0	0	0	0	0	0	0	0

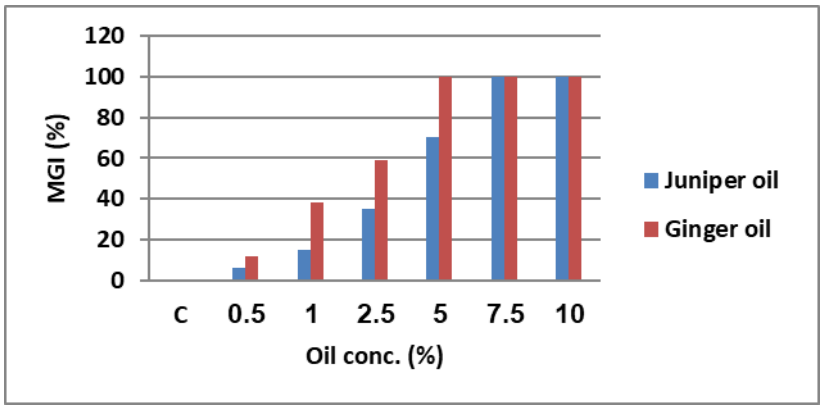

Figure 1. The effects of different concentrations of essential oils on inhibition percentage of mycelial growth of *Aspergillus nigar*

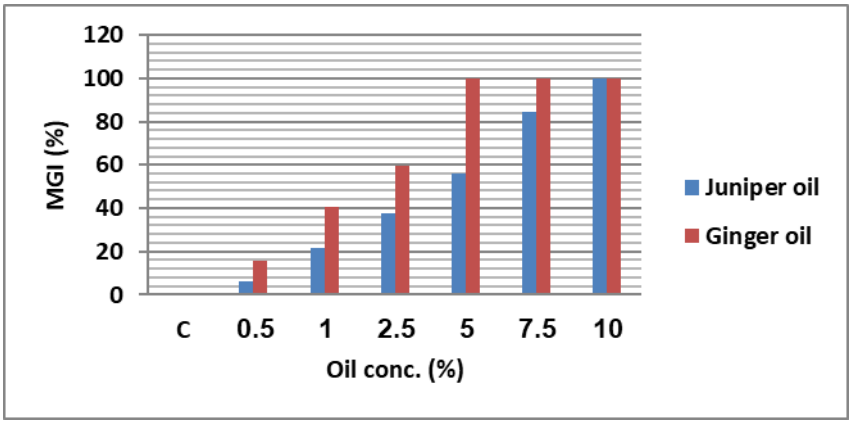

Figure 2. The effects of different concentrations of essential oils on inhibition percentage of mycelial growth of *Aspergillus ochraceus*

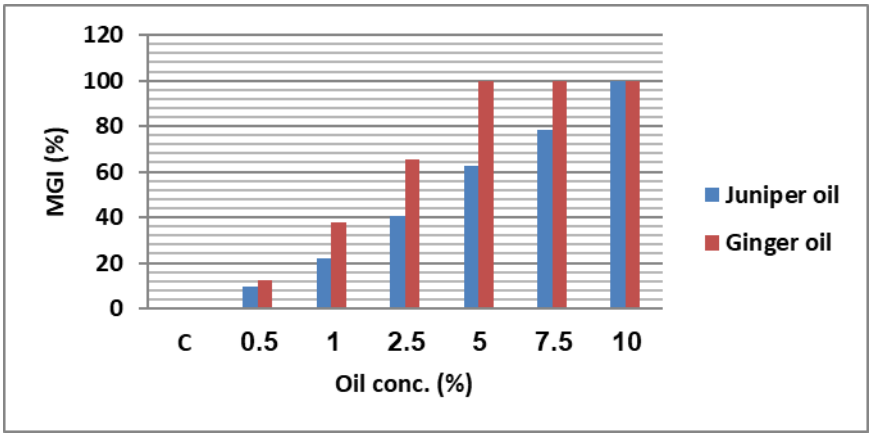

Figure 3. The effects of different concentrations of essential oils on inhibition percentage of mycelial growth of *Aspergillus fumigatus*

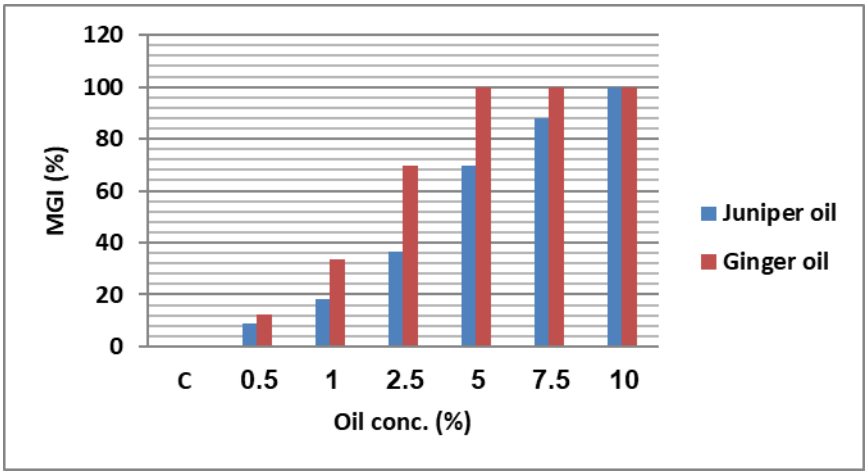

Figure 4. The effects of different concentrations of essential oils on inhibition percentage of mycelial growth of *Aspergillus flavus*

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