Purple basil (Ocimum basilicum var. purple) oil under drought stress effects on Aspergillus fumigates fungus in the food industry

Arghavan Haji Mohammad (Herbal.hmd@gmail.com)
University of Applied Sciences
Hesam Sharifi Nejad
University of Applied Science and Technology

Article

Keywords: essential oils of purple basil, drought stress, Aspergillus fumigates, MFC, MIC90

Posted Date: January 18th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-147652/v1

Purple basil (*Ocimum basilicum var. purple*) oil under drought stress effects on *Aspergillus fumigates* fungus in the food industry

Arghavan Haji Mohammad[1]
1. Msc of Council of Agricultural Engineering herbal.hmd @gmail.com
Hesam Sharifi Nejad[2]
2. Information Technology Engineer (IT)
sharifarsam@gmail.com

Abstract

To investigate the effect of drought stress Purple Basil (*Ocimum basilicum* var.purple), on *Aspergillus fumigates* in food industry, an experiment was performed in the Alborz Karaj research station, and extracted essential oils of purple basil in Agricultural Education Center of Imam Khomeini in 2011, wasperformed. Purple basil essential oil obtained from the stress levels of 30%, 60% and 90% field capacity (FC) is.
Using brows microdilution, the average minimum inhibitory concentration and the mean minimum concentration of essential oils produced in concentrations of 0/25, 0/5, 1, 1/5, 2 and 2/5 mg/ml against fungi *Aspergillus fumigates* was determined in three replicates. The results showed that under conditions of purple basil oil 30% field capacity had the strongest activity against the fungi studied (0/37 mg/ml = 90MIC).Antibacterial activity of essential oil and the lowest was seen in 90% of field capacity (0/97mg/ml = 90MIC). Purple basil oil under the mg/ml) and maximum (0/99 terms of the MFC as the lowest 30% of field capacity (0/56 mg/ml) was seen in 90% of the crop. According to survey results in the absence of purple basil oil 30% field capacity (severe water stress) was a good performance against fungi *Aspergillus fumigates* antifungal and take advantage of it to use its influence in the food industry, pharmaceutical and health is .

Keywords: essential oils of purple basil, drought stress, *Aspergillus fumigates*, MFC, MIC90

Introduction

Chemical drugs and food preservatives have been considered carcinogens, poisoning agents, and defective agents. Consumers are skeptical of chemical additives, and therefore demand for natural and acceptable substances. Society has increased in order to prevent diseases in humans and livestock, food storage and fight pests and rot. Also, with the recent increase in antibiotic-resistant infections, research into drugs with new efficacy against infections is a definite necessity (8).

Essential oils are a mixture of volatile secondary metabolites derived from plants that are extracted in a variety of ways. Essential oils are very complex natural compounds that can contain about 20 to 60 components in completely different concentrations (4). The family Aspergillus fungi is a wide range of fungal diseases caused by members of the genus Aspergillus. The disease may be due to food poisoning, allergies due to inhalation of fungal conidia, or aspergilloma, invasive inflammatory disease of granulomatosis and necrosis of the lungs and other organs, and rarely diffuse visceral fatal disease (9).
The genus Aspergillus includes approximately 900 species, of which Aspergillus fumigatus is the

most isolated agent (12). Red basil is an annual, herbaceous, standing, hairless, fragrant plant with a height of 30 to 70 cm, which belongs to the mint family and has a chromosome number of 48n = 2 (6). Also, water is one of the most important environmental factors that has a major effect on the growth and amount of active ingredients of medicinal plants. (11) Aspargillus fumigatus, which will be a major step in the food and pharmaceutical industries.

In recent years, a large number of essential oils and their components in terms of antimicrobial properties on many bacteria and fungi, and in more than five hundred reports, have been studied (4, 8) in a study that On thyme, savory, mint, lemon, barijeh and eucalyptus on the growth control of Aspergillus parasiticus by natural essential oils in artificial culture medium, it was observed that the minimum inhibitory concentration of thyme (200 ppm) and savory (400 pp) P.M.), both of which had the highest control effect against this fungus (2). Cumin and cockatiel have also been reported to have the antifungal properties of Aspargillus fumigatus (3). Due to the fact that no research has been done on the antifungal properties of purple basil on Aspargillus fumigatus, it is hoped that purple basil in the health industry can be considered a major step in this industry in terms of antifungal properties.

Materials and Methods

This experiment was carried out in the Institute of Forests and Rangelands of the country and Alborz Research Station in Karaj and Imam Khomeini Jihad Agricultural Training Center in 2011. Experiment using a randomized complete block design with three replications in which the main factor consisted of three essential oils under drought stress conditions of 90% (control) 60, 60% (medium stress) and 30% (severe stress) of field capacity and the secondary factor was The minimum concentration (MIC) and inhibitory effect (MFC) were.

After collecting and grinding the mentioned plant materials, their essential oils were prepared by using Clevenger device by distillation with water and then the essential oil was decomposed and after dehydration with waterless sodium sulfate, in brown vials with a volume of 10 Collect ml, and after adding 4 lids of vials, keeping the specifications of the relevant essential oil are stored in the refrigerator until use.
The essential oils used in the study include purple basil essential oil under 30, 60 and 90% FC dry stress conditions and the essential oil is extracted. The standard solution was prepared from essential oils at twice the concentration of the first test concentration. For this purpose, first, dimethyl sulfoxide solvent was used, which was diluted with 0.1 ml of RPMI-1640 liquid culture medium. For instance, to make a stock of 2 mg / ml of essential oil, add 10 µl of pure essential oil from distillation to 0.5 diluted DMSO, and then 4.5 ml of 1640 RPMI. Add the liquid until the stock volume reaches 5 ml, (because by calculation, the concentration of 2 mg / ml is equal to 2 µl / ml, and 10 µl / 5 ml) in this study all The chemicals were purchased from the German company Merck.

The fungal species Aspergillus fumigatus (ATCC-16913) was used as a microorganism. This species was obtained from the fungal collection of Mycology Research Center of the Faculty of Veterinary Medicine, University of Tehran. Fungal species were cultured in potato jellies in a sloping manner at 28 ° C for 10 days. After harvesting from 0.01% of the culture medium, the conidia were placed in sterile distilled water containing 80 tween. Then, they were mixed for 15 seconds and the heavy parts were allowed to settle for 5 minutes. Spores in the resulting suspension were counted by 3 homocytometer slides with 3 replications and standardized at a concentration of 1 10 106 conidia per ml.

To determine the antifungal activity of essential oils, in order to determine the minimum inhibitory concentration and the minimum fungicidal concentration, the macrodilution broth dilution method was used, so that each of the essential oils was first tested experimentally and separately on Aspergillus fumigatus 1 0.25, 0.25, 0.5, 0.75 and 1 mg were affected and using the results, the desired essential oils in concentrations of 0.25, 0.5, 1, 1.5 2, 2.5 and 3 mg / ml were assayed by macrodilution broth on these fungi.

Accordingly, seven erlens containing culture medium, essential oil and conidia of the fungus and seven erlens as control, including 2 positive controls for the fungus and one negative control for each of the essential oils, were considered. Each Erlenmeyer flask contained 100 ml of liquid culture medium for yeast extract. In positive control erlens, the culture medium and fungal spores and in negative control erlens, there was only culture medium and essential oil. Here, the appropriate amount of essential oil was calculated according to the concentration of essential oil tested and the volume of culture medium. After dissolving in 0.5 ml of DMSO solvent was added to the culture medium. For example, to prepare a dilution of 3 mg / ml, 300 µl of essential oil had to be added to E Then, a sufficient amount of standard conidia suspension from the previous step was added to each of the erlens containing the culture medium. The erlens were transferred to a 28 ° C greenhouse with a slow stirrer and the culture result was visually evaluated for growth or non-growth after 48 to 72 hours, and then up to 4 days. In addition, each of the tested samples was replanted, which

The results were recorded and judged on that basis. The final results were calculated as MFC and MIC90 averages from 3 tests, which are shown in Table 1. All equipment, materials and culture media used were sterile and the work was done under the hood, by the flame and with gloves and a mask. And was performed under sterile conditions.

rlenmeyer containing 100 ml of culture medium after dissolving in 0.5 ml of DMSO.

MIC in culture plates is a plate in which a decrease in fungal growth is observed in the concentration of essential oil, compared to the positive control plate. In this study, what is recorded as MIC is MIC90, a plate that shows a 90% reduction in growth compared to a positive control. MFC is a plate in which the concentration of essential oil, and compared to a positive control plate, no growth is possible Fungal observations are not evident even after incubation of replants.

Mean statistics, standard deviation and confidence interval were used to analyze the data. In order to determine the existence or non-existence of differences between two independent means using confidence intervals, the following criteria were considered: 1- The confidence intervals of the two means are completely separate from each other and have no common denominator, therefore, 95% confidence We have that there is a difference. 2. The confidence interval of two averages not only have a common chapter, but the average of one group is in the confidence interval of the other group. Therefore, we conclude with 95% confidence that there is no difference between the two averages.

Research results

The results of essential oil compounds (Table 1) showed that the highest percentage of linalool composition in drought stress was 30% FC and also the highest effect on Aspargillus fumigatus in FC conditions was 30% (5). Mean, standard deviation and assurance of 3 replications of MFC and MIC experiments of purple basil essential oils under 30%, 60% and 90% fc conditions against Aspergillus fumigatus mold by macrodilution broth, after 48 hours Incubation in mg / ml is presented in Table 2.

According to the data in the table above, the average growth inhibitory and fungicidal effects of these essential oils against Aspergillus fumigatus molds depend on the type of essential oil, the amount used (mg / ml) and the fungus under test, so that the lowest values The average MIC and MFC is allocated to purple basil essential oil under stress of 30% of field capacity and the highest amount is related to purple basil essential oil under 90% of field capacity.

According to the findings of this study, the strongest antifungal effect of the tested essential oils is related to the essential oil under 30% fc. The MIC of the vegetable oils tested against the fungus starts at 0.25 mg / ml and ends at 1.5 mg / ml.
The MFC of these oils was also determined to be 0.5 to 2 mg / ml. The calculated safety limits confirm the 95% certainty of the activity and effect of certain amounts of these essential oils against the fungus Aspargillus fumigatus. The mean MFCs and MICs of the tested essential oils were calculated. Finally, the difference in MIC obtained for the essential oils under 30%, 60% and 90% FC conditions were 0.37, 0.69 and 0.98 mg / ml and the difference, respectively. MFC was determined to be 0.56, 0.84 and 0.99 mg / ml, respectively.

Table 1- Comparison of the mean of the amount of purple basil compounds in 1390

3/23b	0/67c	0/92c	0/88c	1/24c	50/46c	0/57c	0/65c	19/92c	1/43c	0/66c	۹۰٪FC
2/19c	0/86b	1/10b	1/26b	1/39b	53/30b	0/73b	0/99b	29/69b	2/53b	1/48b	۶۰٪FC
3/74a	1/03a	1/29a	2/20a	1/96a	55/28a	1/14a	1/27a	31/83a	2/79a	1/90a	۳۰٪FC

The same letters in each column indicate that there is no significant difference between the means

Table 2: Mean, standard deviation and confidence interval (95%) of minimum growth inhibitory concentration (MIC) and minimum lethal concentration (MFC) against Aspargillus fumigatus by concentration

Aspergillus fumigatus						Herbal essential oils
MFC			MIC			
Assurance distance	Standard deviation	Mean	Assurance distance	Standard deviation	mean	
0/86-0/65	0	0/99	0/45-0/35	0	0/98	Basil %90C)essential (F
1/43-0/99	0/39	0/84	0/76-0/65	0/23	0/69	Basil (FC%60)essential
2/2-2/2	0/44	0/56	0/78-1/65	0/34	0/37	Basil (FC%30)essential

Discussion, analysis and conclusion Essential oils from DNA, RNA, proteins and polysaccharides in fungal and bacterial cells They prevent, and these substances cause changes similar to the effects of antifungal activity on fungi. Many researchers pay attention to the antimicrobial function of essential oils and test some of the different components of essential oils and their compounds in order to find possible synergistic effects (4, 8).

In this study, the greater effect of purple basil essential oil can be due to some different compounds, structural form, components and their operating groups and the possibility of synergistic performance between their components that has increased during drought stress. , Linalool, alpha bermogatene, prasimane, etc. (10). Asghari Marjanloo (2008) observed that basil essential oil can replace artificial fungicides in controlling fungal diseases on agricultural products due to its high fungicidal properties, but much research is needed to achieve a suitable formulation. Linalool is a compound found in basil and mint in general, which was found in an experiment to be one of the most effective compounds in controlling Aspargillus (5). According to studies, it has been proven that basil essential oil under 30% FC can prevent the formation of conidia due to increased linalool composition (Haji Mohammad, 2012), which will be a big step towards maintaining health and improving the food industry in society (12).

Reference

1-Asghari Marjanloo A, The effect of basil essential oil on the control of gray rot and quality after strawberry harvest (Selva), Medicinal Plants Quarterly, 28: 131-139.

2- Maskouki A, Mortazavi S, Rad S, 2005. Controlling the growth of Aspargillus parasiticus by natural essential oils in artificial culture medium. Agricultural Sciences and Natural Resources. Volume 3, No 11, pp. 61-70.

3- Minoobayan Haghighi M.H, Khosravi A, 2009. Effects of plant essential oils on Aspargillus species. Quarterly Journal of Gonabad University of Medical Sciences and Health Services. Volume 15, No 4. pp. 5-16.

4- Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils-A review. Food

and Chemical Toxicology 2008; 46: 446-475.

5- Basilico, M.Z., Basilico, J.C. 1999. Inhibitory effects of some spice essential oils on Aspergillus

ochraceus NRRL 3174 growth and ochratoxin A production. Inhibition of ochraceus NRRL. 29(4): 238-241.

6- Bentham, G. 1848. Labiatae in prodomus systematic naturalis regni vegetabilis pars XII, Treuttel and wurtz , Paris , PP: 24 - 608.

7-Dorman HGD and Deans SG. Antimicrobial agents from plants: antibacterial activity of plant

volatile oils. J Appl Microbiol 2000; 88(5): 308-316.

8- Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils (A review article).

Current Medicinal Chemistry 2003; 10: 813-829.

9- Kwon – Chung KJ, Bennett JE. Medical mycology. 2nd ed. L ea & Febrger. Philadelphia 1992, pp: 201 - 10.

10- said al ahl M.S. Hussein. 2010. Eefect of water stress and potassium on the productivity of oregano plant using saline and fresh water irrigation. Ozean journal of applied sciences. 3(1): 125 - 141

11- Wagner T, 1993. Chamomile production in Slovenia. Acta Horticulturae 344: 476 -478.

4- Zeini F, Basiri Jahromi S H. Study of fungal infections in patients withLeukemia. Iran. J. Pub heal. 1994; 1 (4): 89 - 103.

12- Haji Mohammad A, 2012. Comparission of essential oil yield and composition of three popilation of purple basil (Ocimum Basilicum var.purple)