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Research Article

Determination of the Potential for use of Plant Essential Oils as a Fungicide Against *Fusarium Oxysporum* (OG10)

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Abstract

This study aimed to determine whether the essential oils of thyme, ginger, and mint from medicinal aromatic plants can provide resistance to the pathogen *Fusarium oxysporum* in the maize plant. To this end, the antifungal effect of 0.1 ml, 0.25 ml, 0.5 ml, and 1 ml essential oil amounts was determined by the agar disc diffusion method. It was determined that concentrations containing 0.1, and 0.25 ml essential oil showed no antifungal effects, however, concentrations containing 0.5 and 1 ml essential oil had antifungal effects. The most effective concentration was found to be 1 ml of essential oil in all three species. The maize was grown under hydroponic conditions. Thyme, ginger, and mint essential oils (1 g/100 ml) were applied to the root medium of the grown maize plant on the 8th day. An *F. oxysporum* suspension containing 107 spores was applied after 24 hours and harvested 3 days later. When the reactive oxygen species (H_2O_2) and MDA amounts of the harvested plants were examined, it was observed that there was an increase in the population of *F. oxysporum*. However, applications of thyme, ginger, and mint essential oil have been observed to significantly reduce these. It was also determined that essential oils protected the plant against *F. oxysporum* by increasing antioxidant enzyme activities. Although these three essential oils applied have antifungal properties, it has been observed that the best effect belongs to thyme essential oil. The results show that essential oils of thyme ginger and mint can be used as potential fungicides against the pathogen *F. oxysporum* in maize cultivation

More Information

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Introduction

Due to the rapid increase in the world population, the need for food is also increasing. Meeting this basic need is possible by increasing the amount of production and by increasing the amount of product to be obtained from the unit area. This is due to the selection of resistant and high-yielding plants, fertilization and irrigation, as well as the cultivation of the product to be obtained free of diseases, pests, and weeds. Fungi, which are plant pathogens, enter plant tissues in various ways, sometimes from stomata and sometimes from roots. Plant-pathogenic fungal diseases cause crop losses both in the field and in storage. Synthetic fungicides are used to prevent these losses. The unconscious use of these fungicides for many years causes resistance to pathogens, as well as the accumulation of toxic substances in water, air, and soil. Therefore, it harms human and animal health [1-3]. New antifungal agents are sought that do not harm nature and do not adversely affect human health. One of these substances is the use of herbal products that are environmentally friendly, do not have residue problems, and protect plants from diseases and pests. Plants have rich content. Various studies have determined that especially essential oils and secondary metabolites can control diseases, pests, and weeds [4-6]. Since plant secondary metabolites and essential oils contain various bioactive substances, they have the potential to be used as new and natural antifungals [7].

In particular, plant essential oils are more effective and more useful [8]. Thyme, ginger, and mint herbs, which are used in spices and traditional medicine, are rich in essential oils and secondary metabolites. It has been determined in various studies that the essential oils of these plants have antifungal effects [8-12].

It has been determined that these plant essential oils have antifungal properties against various plant pathogenic fungi. One of them is *Fusarium* sp. their antifungal effect. *Fusarium* causes disease in various cereals and plants with economic value. One of these plants is corn, which is cultivated in the world and consumed as human and animal food rich in carbohydrates [13,14]. *Fusarium*, which causes root rot, stem, and cob rot in maize, causes great economic losses.

This study is an attempt to determine the antifungal effects of the essential oils of dried thyme, mint, and ginger plants against *Fusarium oxysporum*, which causes root rot in maize cultivated in our country and in the world with high economic and nutritional properties, and the effect on the antioxidant system in maize. This is the first study to investigate the effect of *F. oxysporum* on the antioxidant system in maize.

Material and methods

Development of the fungus

The fungus used in the study was obtained from the culture collection of Atatürk University, Faculty of Science, Department of Biology, Microbiology Laboratory. The accession number of *F. oxysporum* with code OG10 is OQ975306. *F. oxysporum* culture inoculated on Potato Dextrose Agar (PDA) was incubated at 28 C for 7 days. A prepared 5 ml spore suspension was then placed in 100 ml potato dextrose broth (PDB) in 250 Erlenmeyer and incubated at 28 °C at 180 rpm for 96 hours.

Application of *Fusarium oxysporum* to maize seedlings

Maize (*Zea mays* L. Arifiye-2) seed was grown in a hydroponic medium. Seeds and pots were sterilized with 10% sodium hypochlorite and 95% ethanol for 5 minutes. Seed germination and seedling growth were carried out under control conditions at 24/22 °C, 70% humidity, and 16/8 hours light-dark period.

For each pot (in 10 L volüme), 100 ml of mycelium was placed in 100 ml of isotonic water and the micelles were broken up with micelles. Then it was applied to the roots of 9-day-old *Zea mays* seedlings. 24 hours after mycelial application, on day 15, the plants were harvested, root, stem lengths, and weights were taken, and the plant parts were stored at -18 °C for further analysis after lyophilization in liquid nitrogen.

Obtained of crude essential oils

For obtaining crude essential oils; 500gm of thyme, mint, and ginger herbs were weighed and powdered. Plant samples were placed in sterile distilled water and steamed (5 v/W). It was then distilled under atmospheric pressure. The distillate was centrifuged at 3,000 rpm for 3 minutes [15].

Agar well diffusion method

0.1, 0.25, 0.5 ml and 1 ml essential oil were placed in potato dextrose agar medium and 1 cm^2 active fungal cultures (4 days culture developed on PDA) were placed in the middle of solidified agar. It was incubated at 25 °C. The developmental stages were monitored, the 3^{rd}-day and 7^{th}-day stages were recorded and the antifungal activity of plant essential oils was determined [16,17].

Determination of lipid peroxidation

Lipid peroxidation (LPO) change was estimated by measurement of malondialdehyde (MDA) content according to Heath and Packer 1968 [18]. For this, the bean leaves (0.5 g) were homogenized in 5 ml of cold TCA (5%) and then the obtaining homogenate was centrifuged at 12,000×*g* for 15 min at +4 °C. One ml of 0.5% thiobarbituric acid (TBA) prepared in TCA (20%) was mixed with 4 ml of the supernatant in a clear tube. After the mixture was incubated in boiling water for 30 min, the reaction was stopped by removing the tubes in an ice bath. The tubes were centrifuged again at 10,000×*g* for 10 min. The absorbance of the supernatant was monitored at 532 nm and then non-specific absorption at 600 nm was determined. For the calculation of lipid peroxidation, the absorbance value measured at 532 nm was subtracted from the value determined at 600 nm and calculated in a 1 ml solution of MDA (nmol/ml): by the formula $[(A532\text{-}A600)/155000] \times 10^6$. Data are submitted as MDA (nmol/g tissue) [19].

Determination of hydrogen peroxide (H_2O_2) content

The H_2O_2 content was determined by using the method described by He, et al. 2005 [20]. Fresh leaves (0.5 g) were homogenized in 10 ml of cold acetone and the homogenate was centrifuged at 10,000×*g* for 10 min at +4 °C. Then, 0.15 ml of 5% $Ti(SO_4)_2$ and 0.3 ml of NH_4OH were added to 1.5 ml of supernatant. After the precipitation, the mixture was further centrifuged at 10,000×*g* for 10 min. After removal of the supernatant, the obtained pellet was dissolved in 3 ml of 2 M H_2SO_4, and its absorbance was measured at 415 nm. These absorbance values were converted to the amount of H_2O_2 in the nanogram through a standard graph. Data are submitted as the amount of H_2O_2 per g leaf (ng/g leaf).

Determination of the activity of antioxidant enzymes

The activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and antioxidant enzymes were determined spectrophotometrically in the cellular fluid obtained from maize leaves. SOD activity was monitored by measuring its ability to reduce the photochemical reduction of nitro blue tetrazolium (NBT). POX activity was measured based on the rate of decomposition of H_2O_2 by peroxidase with guaiacol as a hydrogen donor at 470 nm. One unit of POX is defined as the amount of enzyme that causes a 0.01 increase in absorbance per minute [19].

Statistical analysis

All experiments were performed 6 times and the average of values was presented. The data were analyzed by analysis of variance, and means were compared by using the Tukey-Kramer Test at < 0.05 significance level.

Results

In this study, the antifungal effects of different concentrations of essential oils were tested. The antifungal effect of 0.1, 0.25, 0.5 ml and 1 ml (1 mg/ml) essential oil on *F. oxysporum* was measured on the 3^{rd} and 7^{th} days. However, no effect was observed at 0.1 and 0.25 ml essential oil concentrations. For this reason, the results are not shown in Table 1. The antifungal effect of 0.5 and 1 ml of essential oils on *F. oxysporum* was determined according to the measured zone diameters. It inhibited growth at both concentrations compared to the control, but 1 ml of essential oils was found to be more effective.

Table 1: The antifungal effect of the obtained crude essential oils on *F. oxysporum* at different concentrations was determined by measuring the zone diameters.

Applications	3rd day (0.5 ml)	7th day (0.5 ml)	3rd day (1 ml)	7th day (1 ml)
Control	3.5 cm	7 cm	3.5 cm	7 cm
Thyme essential oil	1.6 cm	2.3 cm	-	-
Ginger essential oil	1.5 cm	2.5 cm	-	-
Mint essential oil	1.5 cm	3 cm	-	-

Since 1 ml essential oils are more effective in determining the antifungal effect, 1 ml concentration was chosen in plant trials and the results were given based on 1 ml.

In the application of *F. oxysporum* to maize seedlings; the amount of H_2O_2, which is one of the reactive oxygen species, increased compared to the control (102 µg/g-1). A decrease in the amount of H_2O_2 was observed in essential oils with antifungal effects (Figure 1).

F. oxysporum application increased the MDA amount by 106% compared to control. When the essential oils we apply are compared with the *F. oxysporum* application; Thyme, mint, and ginger decreased MDA by 24%, 3.2% and 14%.

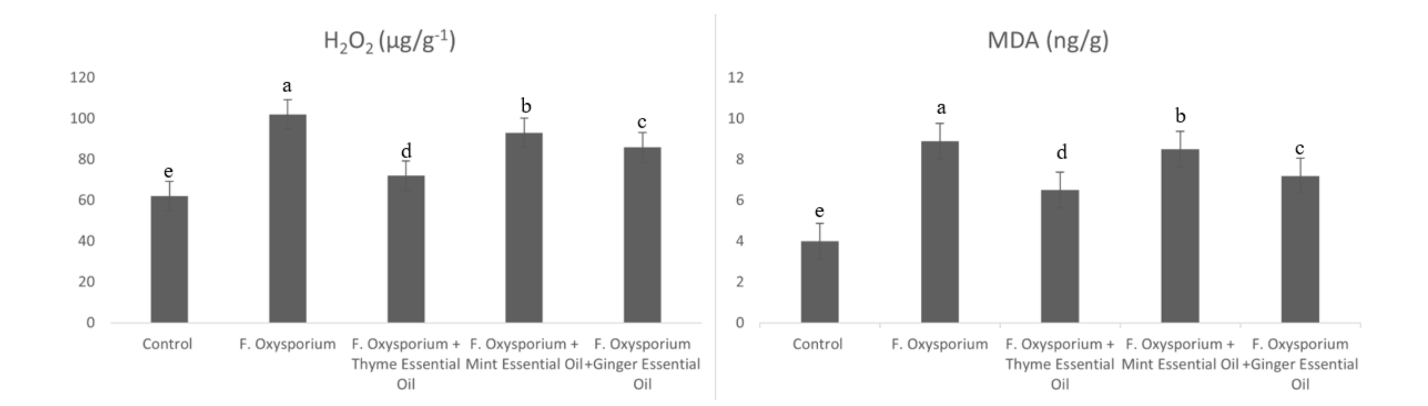

Figure 1: The amounts of H_2O_2 and MDA. Different letters indicate a significant difference at $p < 0.05$ significance level, provided that the groups are among themselves. Bars mean standard error.

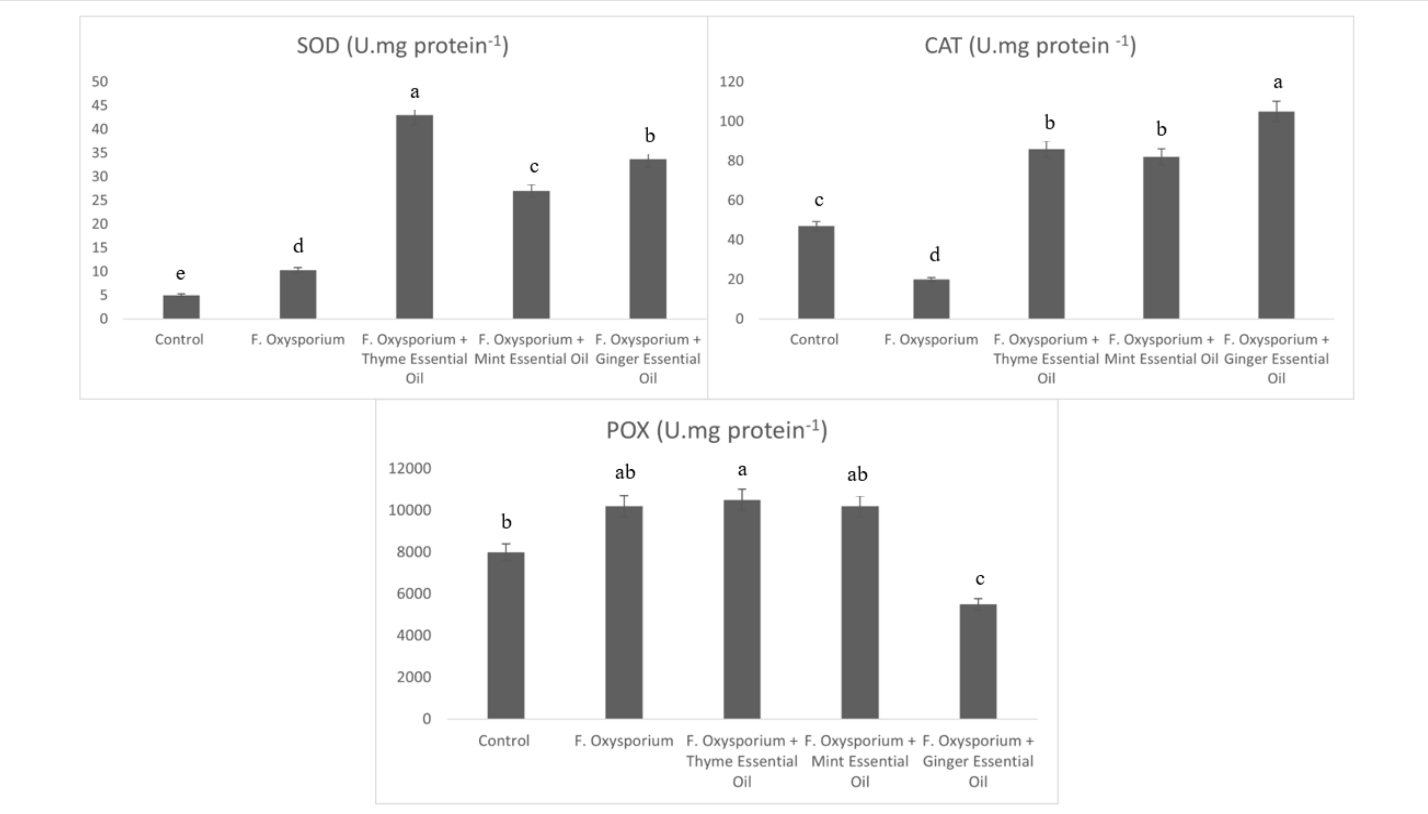

Figure 2: Antioxidant enzyme activities of essential oils applied to maize plant against *F. oxysporum*. Different letters indicate a significant difference at $p < 0.05$ significance level, provided that the groups are among themselves. Bars mean standard error.

Antioxidant enzymes, which are an integral part of the antioxidant defense system, have a vital role in clearing the ROS produced in plant cells. It was observed that thyme, mint, and ginger essential oils increased SOD activity by 33%, 18% and 24%, respectively, compared to *F. oxysporum*. It was observed that while *F. oxysporum* application decreased CAT activity compared to control, three essential oil applications significantly increased CAT activity it observed that while POX activity increased with the application of thyme and mint essential oil, it decreased the activity with the application of only ginger essential oil (Figure 2).

Discussion

Maize (*Zea mays*) is among the most cultivated plants in the world with high nutritional and economic value. As there are microorganisms that cause diseases in all plants, there are also microorganisms that cause disease in corn plants. Among these organisms, fungi, especially *Fusarium* species, stand out. Many fungicides with chemical content are used to prevent the damage caused by *Fusarium* species. However, the use of these fungicides harms nature and human health. In order to eliminate or minimize this damage, alternative antifungal agents that do not harm the environment are sought. Among these alternatives, there is an increase in the use of biodegradable, short-lived plant secondary metabolites, especially plant essential oils [21-23]. In this context, essential oils of thyme, ginger, and mint plants were used as antifungal agents against *F. oxysporum* in our study. The antifungal properties of the essential oils of these plants were determined by the agar disc diffusion method. The essential oils obtained were placed in 25 ml PDA as 0.5 ml and 1 ml. The antifungal effect on *F. oxysporum* was followed for 3 and 7 days and the zone diameters were measured (Table 1). While there was an improvement compared to control (7 cm) at 0.5 ml essential oil concentration (thyme 2.3cm, ginger 2.5cm, and mint 3cm), there was no improvement at 1 ml concentration. The antifungal properties of essential oils have been determined in previous studies. Thanks to the antioxidant substances contained in thyme, ginger, and mint plants, they support the defense systems of the plants. In the studies, it was found that these essential oils have antifungal properties against *Fusarium* and it is seen that our study overlaps with the literature [23-25].

Reactive oxygen species (ROS), one of the plant defense system responses to various biotic or abiotic stresses, are hydrogen peroxide: H_2O_2, superoxide: O_2^- and hydroxyl radical: OH^-. ROS causes cell fragmentation (increase in the amount of MDA), DNA damage and ultimately plant death. Pathogenic or non-pathogenic fungi that infect the plant cause an increase in reactive oxygen species in plants [26-28]. Plants have developed antioxidant systems to eliminate or minimize ROS damage. Superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) enzymes are included in this system [29,30]. This is the first detailed study on the ROS damage caused by *F. oxysporum* in maize plants and the antioxidant system, which is the defense mechanism to eliminate this damage. In this study, the amount of MDA and H_2O_2 increased in the application of *F. oxysporum* compared to the control, and it was observed that the essential oils were significantly reduced in the amounts of MDA and H_2O_2 through the antifungal compounds they contain (Figure 1). In particular, thyme essential oil reduced the amount of MDA and H_2O_2 by 75% on average. Previous studies stated that fungi increase the amount of ROS and cause the cell structure of plants to deteriorate and die [31,32]. It has been emphasized in many studies that plant essential oils reduce the accumulation of ROS caused by fungi with the antifungal compounds they contain [33-36]. Our study overlaps with the literature and it is thought that antioxidant enzyme activities have an effect on it. In many studies, it has been found that plant essential oils with antifungal properties contribute to plant defense by increasing the amount and activities of SOD, CAT, and POX enzymes [28,35,36]. As shown in Figure 2, the essential oils used against *F. oxysporum* activate the defense system in the corn plant, enabling the plant to survive and gain protection against pathogens.

Maize is a cultivated plant with nutritional and economic value. Such an important plant suffers from high yield losses every year by fungal pathogens. Many chemical fungicides are used to prevent yield loss. It is anticipated that the use of these fungicides will adversely affect the production of future maize and other agricultural crops, human and animal health, and the environment. Considering these effects, new fungicides are being sought that are eco-friendly, inexpensive to use, obtain, and cost. Based on the results of our study, the usability of thyme, mint, and ginger essential oils, which we think will contribute to this search, has been demonstrated. Considering this study, the use of plant essential oils as fungicides in plant agriculture should be increased and supported.

Conclusion

The antifungal effect of essential oils of medicinal aromatic plants has been determined and for this purpose; essential oils of thyme, mint, and ginger plants were obtained. Concentrations of 0.1, 0.25, 0.5, and 1 ml were tried and no antifungal effect was found at lower doses. However, they have been determined that 0.5 and 1 ml of essential oils have an antifungal effect. The dose with the highest antifungal effect (1 ml) was tested in plant experiments and was determined to make the plant resistant to root rot in maize plants. With the results obtained, it was determined that these essential oils could be used as antifungal agents against *F. oxysporum*.

Author contributions: Each author contributed equally.

References

1. Hahn M. The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study. J Chem Biol. 2014 May 28;7(4):133-41. doi: 10.1007/s12154-014-0113-1. PMID: 25320647; PMCID: PMC4182335.

2. Shuping DSS, Eloff JN. The use of plants to protect plants and food against fungal pathogens: a review. Afr J Tradit Complement Altern Med. 2017 Jun 5;14(4):120-127. doi: 10.21010/ajtcam.v14i4.14. PMID: 28638874; PMCID: PMC5471458.
3. Hamad YK, Abobakr Y, Salem MZ, Ali HM, Al-Sarar AS, Al-Zabib AA. Activity of plant extracts/essential oils against three plant pathogenic fungi and mosquito larvae: GC/MS analysis of bioactive compounds. BioResources, 2019; 14(2): 4489-4511.
4. Kaur R, Kaur H. The Antimicrobial activity of essential oil and plant extracts of Woodfordia fruticosa. Arch Appl Sci Res. 2010; 2(1): 302-309.
5. Hussain AI, Anwar F, Shahid M, Ashraf M, Przybylski R. Chemical composition, and antioxidant and antimicrobial activities of essential oil of spearmint (Mentha spicata L.) from Pakistan. Journal of Essential Oil Research. 2010; 22(1): 78-84.
6. Douiri LF, Boughdad A, Alaoui MH, Moumni M. Biological activity of Rosmarinus officinalis essential oils against Callosobruchus maculatus, (Coleoptera, Bruchinae). Journal of Biology Agriculture and Healthcare. 2014; 4(2): 5-14.
7. Matoušková M, Jurová J, Gruľová D, Wajs-Bonikowska A, Renčo M, Sedlák V, Poráčová J, Gogaľová Z, Kalemba D. Phytotoxic Effect of Invasive Heracleum mantegazzianum Essential Oil on Dicot and Monocot Species. Molecules. 2019 Jan 24;24(3):425. doi: 10.3390/molecules24030425. PMID: 30682808; PMCID: PMC6384721.
8. Elshafie HS, Camele I. An Overview of Metabolic Activity, Beneficial and Pathogenic Aspects of Burkholderia Spp. Metabolites. 2021 May 17;11(5):321. doi: 10.3390/metabo11050321. PMID: 34067834; PMCID: PMC8156019.
9. Ibáñez MD, Blázquez MA. Ginger and Turmeric Essential Oils for Weed Control and Food Crop Protection. Plants (Basel). 2019 Mar 10;8(3):59. doi: 10.3390/plants8030059. PMID: 30857365; PMCID: PMC6473496.
10. Guo J, Gao Z, Li G, Fu F, Liang Z, Zhu H, Shan Y. Antimicrobial and antibiofilm efficacy and mechanism of essential oil from Citrus Changshan-huyou YB chang against Listeria monocytogenes. Food Control. 2019; 105: 256-264.
11. Abdollahi M, Sefidkon F, Calagari M, Mousavi A, Mahomoodally MF. Impact of four hemp (Cannabis sativa L.) varieties and stage of plant growth on yield and composition of essential oils. Industrial Crops and Products. 2020; 155: 112793.
12. Hu Z, Yuan K, Zhou Q, Lu C, Du L, Liu F. Mechanism of antifungal activity of Perilla frutescens essential oil against Aspergillus flavus by transcriptomic analysis. Food control. 2021; 123: 107703.
13. Okello PN, Petrović K, Kontz B, Mathew FM. Eight species of Fusarium cause root rot of corn (Zea mays) in South Dakota. Plant Health Progress. 2019; 20(1): 38-43.
14. Ngoune Tandzi L, Mutengwa CS. Estimation of maize (Zea mays L.) yield per harvest area: Appropriate methods. Agronomy. 2020; 10(1): 29.
15. Matsumura S, Murata K, Zaima N, Yoshioka Y, Morimoto M, Kugo H, Yamamoto A, Moriyama T, Matsuda H. Inhibitory Activities of Essential Oil Obtained from Turmeric and Its Constituents against β-Secretase. Nat Prod Commun. 2016 Dec;11(12):1785-1788. PMID: 30508333.
16. Castillo F, Hernández D, Gallegos G, Mendez M, Rodríguez R, Reyes A, Aguilar CN. In vitro antifungal activity of plant extracts obtained with alternative organic solvents against Rhizoctonia solani Kühn. Industrial Crops and Products. 2010; 32(3): 324-328.
17. Kim E, Park IK. Fumigant antifungal activity of Myrtaceae essential oils and constituents from Leptospermum petersonii against three Aspergillus species. Molecules. 2012 Sep 3;17(9):10459-69. doi: 10.3390/molecules170910459. PMID: 22945026; PMCID: PMC 6268886.
18. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 1968 Apr;125(1):189-98. doi: 10.1016/0003-9861(68)90654-1. PMID: 5655425.
19. Tiryaki D, Aydın İ, Atıcı Ö. Psychrotolerant bacteria isolated from the leaf apoplast of cold-adapted wild plants improve the cold resistance of bean (Phaseolus vulgaris L.) under low temperature. Cryobiology. 2019 Feb;86:111-119. doi: 10.1016/j.cryobiol.2018.11.001. Epub 2018 Nov 10. PMID: 30419217.
20. He Y, Liu Y, Cao W, Huai M, Xu B, Huang B. Effects of salicylic acid on heat tolerance associated with antioxidant metabolism in Kentucky bluegrass. Crop Science. 2005; 45(3): 988-995.
21. Xing F, Hua H, Selvaraj JN, Zhao Y, Zhou L, Liu X, Liu Y. Growth inhibition and morphological alterations of Fusarium verticillioides by cinnamon oil and cinnamaldehyde. Food Control. 2014; 46:343-350.
22. Hassanien MF, Assiri AM, Alzohairy AM, Oraby HF. Health-promoting value and food applications of black cumin essential oil: an overview. J Food Sci Technol. 2015 Oct;52(10):6136-42. doi: 10.1007/s13197-015-1785-4. Epub 2015 Mar 27. PMID: 26396361; PMCID: PMC4573164.
23. Kumar P, Mishra S, Kumar A, Sharma AK. Antifungal efficacy of plant essential oils against stored grain fungi of Fusarium spp. J Food Sci Technol. 2016 Oct;53(10):3725-3734. doi: 10.1007/s13197-016-2347-0. Epub 2016 Oct 13. PMID: 28017987; PMCID: PMC5147695.
24. Sharma A, Rajendran S, Srivastava A, Sharma S, Kundu B. Antifungal activities of selected essential oils against Fusarium oxysporum f. sp. lycopersici 1322, with emphasis on Syzygium aromaticum essential oil. J Biosci Bioeng. 2017 Mar;123(3):308-313. doi: 10.1016/j.jbiosc.2016.09.011. Epub 2016 Nov 18. PMID: 27876218.
25. Katyayani KKS, Bindal S, Yaddanapudi S, Kumar V, Rana M, Srivastava S. Evaluation of bio-agents, essential oils, and chemicals against Fusarium wilt of tomato. Int J. Curr Microbiol App Sci. 2019; 8(07): 1913-1922.
26. Heller J, Tudzynski P. Reactive oxygen species in phytopathogenic fungi: signaling, development, and disease. Annu Rev Phytopathol. 2011;49:369-90. doi: 10.1146/annurev-phyto-072910-095355. PMID: 21568704.
27. Taheri P, Kakooee T. Reactive oxygen species accumulation and homeostasis are involved in plant immunity to an opportunistic fungal pathogen. J Plant Physiol. 2017 Sep;216:152-163. doi: 10.1016/j.jplph.2017.04.018. Epub 2017 Jun 1. PMID: 28667882.
28. Segal LM, Wilson RA. Reactive oxygen species metabolism and plant-fungal interactions. Fungal Genet Biol. 2018 Jan;110:1-9. doi: 10.1016/j.fgb.2017.12.003. Epub 2017 Dec 7. PMID: 29225185.
29. Samoilova Z, Smirnova G, Muzyka N, Oktyabrsky O. Medicinal plant extracts variously modulate susceptibility of Escherichia coli to different antibiotics. Microbiol Res. 2014 Apr;169(4):307-13. doi: 10.1016/j.micres.2013.06.013. Epub 2013 Jul 31. PMID: 23916388.
30. Marroquin-Guzman M, Hartline D, Wright JD, Elowsky C, Bourret TJ, Wilson RA. The Magnaporthe oryzae nitrooxidative stress response suppresses rice innate immunity during blast disease. Nat Microbiol. 2017 Apr 18;2:17054. doi: 10.1038/nmicrobiol.2017.54. PMID: 28418377.
31. Yan L, Li M, Cao Y, Gao P, Cao Y, Wang Y, Jiang Y. The alternative oxidase of Candida albicans causes reduced fluconazole susceptibility. J Antimicrob Chemother. 2009 Oct;64(4):764-73. doi: 10.1093/jac/dkp273. Epub 2009 Aug 5. PMID: 19656781.
32. Mello EO, Ribeiro SF, Carvalho AO, Santos IS, Da Cunha M, Santa-Catarina C, Gomes VM. Antifungal activity of PvD1 defensin involves plasma membrane permeabilization, inhibition of medium acidification, and induction of ROS in fungi cells. Curr Microbiol. 2011 Apr;62(4):1209-17. doi: 10.1007/s00284-010-9847-3. Epub 2010 Dec 19. PMID: 21170711.
33. Tian J, Ban X, Zeng H, He J, Chen Y, Wang Y. The mechanism of antifungal action of essential oil from dill (Anethum graveolens L.) on Aspergillus flavus. PLoS One. 2012;7(1):e30147. doi: 10.1371/

journal.pone.0030147. Epub 2012 Jan 17. PMID: 22272289; PMCID: PMC3260232.

34. Shen Q, Zhou W, Li H, Hu L, Mo H. ROS Involves the Fungicidal Actions of Thymol against Spores of Aspergillus flavus via the Induction of Nitric Oxide. PLoS One. 2016 May 19;11(5):e0155647. doi: 10.1371/journal.pone.0155647. PMID: 27196096; PMCID: PMC4872997.

35. Qu S, Yang K, Chen L, Liu M, Geng Q, He X, Li Y, Liu Y, Tian J. Cinnamaldehyde, a Promising Natural Preservative Against Aspergillus flavus. Front Microbiol. 2019 Dec 18;10:2895. doi: 10.3389/fmicb.2019.02895. PMID: 31921070; PMCID: PMC6930169.

36. Lee JE, Seo SM, Huh MJ, Lee SC, Park IK. Reactive oxygen species mediated-antifungal activity of cinnamon bark (Cinnamomum verum) and lemongrass (Cymbopogon citratus) essential oils and their constituents against two phytopathogenic fungi. Pestic Biochem Physiol. 2020 Sep;168:104644. doi: 10.1016/j.pestbp.2020.104644. Epub 2020 Jun 25. PMID: 32711777.