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The fungicidal effect of sesame essential oil against locally isolated fungi from Al Baha and Baljurash regions

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Abstract. Many fungal strains are invading the plants in the agricultural regions and using chemical-based fungicides may cause harm to the plants the then damage the crops. This study aims to assess the fungicidal effect of the composited sesame oil against locally isolated fungi in Al Baha and Baljurash regions in Saudi Arabia. The isolation process focused on infested fields with fungal infection in 11 regions cultivated with olive plants in Al Baha and Baljurash regions, which were then isolated on Potato Dextrose Agar medium for 7 days at 25 C, to be then identified on the genotypic level using ITS genetic marker suitable chromosomal extraction kits and construct the phylogenetic trees by MEGA 11 software. The major 11 fungal pathogens were belonged to *Aspergillus niger, Fusarium equiseti, Alternaria tenuissima, Phoma macrostoma, Alternaria alternata*, and *Aspergillus flavus.* While only one nonpathogenic fungal strain was isolated (*Trichoderma harzianum*). The antifungal activity of the sesame oil in two concentrations was assessed against the isolated fungal strains, the extracted sesame oil with 20 ml concentration showed a promising activity when compared to plain PDA and 10 ml concentration (90.96 to 2.05 mm). In conclusion, sesame oil with maximum concentration can reveal strong fungicidal activity when compared to commercial fungicides and zinc ferrite nanoparticles, therefore, several antifungal approaches must be implemented to combat fungal infections in Al Baha region.

1 Introduction

The Kingdom of Saudi Arabia possesses a diverse and abundant flora, there are around 2300 plant species belonging to 142 families in Saudi Arabia. Olea europaea L, also known as the Olive, is a perennial, arboreal plant that is widely cultivated worldwide, especially in areas with a Red Sea climate.

Some noteworthy fungi that have been documented as invaders in olive plants include *A. alternata,* various species of *Colletotrichum*, *Verticillium dahliae*, numerous species of Fusarium such as *Fusarium oxysporum*, and *Rhizoctonia solani* [1].

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Agricultural pesticides are chemical substances employed to eradicate crop pests or impede the growth or detrimental impacts of these organisms. Fungicides are a class of pesticides that consist of physical, chemical, or biological compounds used to fight against fungal germs [2]. These are extensively employed in agricultural systems to manage illnesses and safeguard the productivity and quality of crops [3].
Sesame seed oil is a widely used edible oil in certain regions such as North India, Iran, Saudi Arabia, and Japan [4]. Sesame cultivation dates back over 5000 years and is commonly grown for its seeds and leaves [5]. Sesame oil has been found to possess antifungal and antibacterial properties in fungal-infected plants and crops [3], its high level of safety and significant fungicidal effects with a large safety index to humans and plants made this edible oil a promising agent to be used against several plants' infections [6].
Developing novel fungicides with desired attributes, such as broad-spectrum efficacy, improved bioavailability, and minimal toxicity and side effects, is challenging because fungal and mammalian cells share similarities in DNA biosynthetic pathways and chromatin organization. As a result, the progress in developing drugs to combat invasive fungal diseases has been sluggish. However, most of these chemicals have been limited in their use in agriculture mostly because they are harmful to plants. Therefore, this study aims to assess the fungicidal effect of the composited sesame oil against locally isolated fungi in Al Baha and Baljurash regions in Saudi Arabia.

2 Materials and Methods

Samples Collection Sites and Sampling
From different 132 samples of the Olive crops were collected from 11 infested sites across Al Baha and Baljurashi regions, and all samples were stored at 4 °C for further studies.
Isolation of Fungi and Stock Culture Preparation
The fungal isolates were isolated and sub-cultured on Potato Dextrose Agar (PDA) Medium at room temperature (25 °C) for 7 days after treatment with 1% sodium hypochlorite solution, at three different media conditions; (first untreated medium = negative control; second medium treated with fungicide; and the third medium treated with plant extract).
A total of 12 fungal strains were confirmed, as the 11 major fungal pathogens + 1 nonpathogenic species in all tested olive fields. About 11 isolates were pathogenic and one nonpathogenic fungal strain was isolated (*Trichoderma harzianum*). For short-term preservation, fungal isolates were maintained on PDA slants, kept at 4ºC and sub-cultured every month.
Detection of fungi' DNA by endpoint PCR
Chromosomal DNA was extracted from the selected fungal isolates using Quick DNA Fungal/Bacterial Miniprep Kit Zymo Research (Epigenetics, California, USA) according to manufacturer's instructions and stored at -70°C for PCR amplification.
Identification of fungi isolates
After the extraction of DNA, GeneJET™ purification kit was used for the purification of PCR products at Sigma Scientific Services Company (Korea). Selected PCR product of fungi was sequenced at GATC Biotech Company in Germany using ABI 3730 xl DNA Sequencer. The alignment and assembly of the obtained forward and reverse sequences into the final consensus was done using BioEdit v7.2.5 software (http://www.mbio.ncsu.edu/BioEdit/). The open reading frames (ORF) of the final contigs for the tested gene were detected by FramePlot 2.3.2. The sequencing data were analyzed using the basic local alignment search tool (BLASTn), and about 12 fungal isolates were successfully identified by molecular genetics approaches.
Extraction of sesame oil

Sesame seeds were acquired, and sesame seed oil was extracted using the Soxhlet extraction process. The sesame seeds were dried naturally for 2 weeks and then pulverized into a fine powder using a grinder. 20 grams of powders were combined with 100 millilitres of pure N-hexane and 100 millilitres of methanol (80% volume/volume). The hexane oil was obtained using a Soxhlet extraction device from Sigma-Aldrich in Germany. The methanol extracts were filtered using Whatman No 1 filter sheets (Whatman, USA) and concentrated using an EYELA rotary evaporator (N-1000, Japan). The concentrated extracts were then freeze-dried using the Edwards freeze dryer (Edwards High Vacuum International Crawley, Sussex, England). A stock solution with a concentration of 10% was created by diluting dimethyl sulfoxide in Roswell Park Memorial Institute (RPMI 1640, Sigma, Germany) for the oil materials, then adding 10 ml and 20 ml of oil on 400 ml of PDA media [7].

Evaluation of Fungicidal activity by diffusion assay

The fungal isolates were sub-cultured on PDA at 25 °C for 48 h and used to evaluate the studied products of two concentrations of extracted sesame oil (10 and 20 mg/ml), as fungicidal against them.

In a diffusion assay, 0.1 mL (10^6 CFU/ml, 0.5 McFarland) inoculum suspension for each isolate was inoculated on the Nutrient Agar (NA) medium to be isolated in a pure form.

Four products were used to be tested against the selected fungal isolates:

- Potato dextrose agar (PDA) medium was used as a negative control for fungal isolates (10^6 CFU/ml), in addition to hexane, and H_2O.
- Tolex 500 WP was used as a positive control (4 gm of fungicide on (1000 ml PDA media).
- Two concentrations of Zn Ferrite nanoparticles (500 and 1000 mm) as positive control.
- Two concentrations of prepared sesame oil as plant extract (10 and 20 ml).

As well as DMSO was used as a control to alleviate the fungicidal effect of it in other formulations. The inhibition of fungal isolates was determined.

The fungicidal effect was performed on the highly susceptible fungal strains to sesame oil [8], following the incubation period, the number of colonies was quantified to assess the bactericidal or fungicidal inhibitory effects using the provided equation:

The fungicidal Inhibitory activity (%) = C-T/C×100.

C is the number of counted colonies as control.

T is the number of obtained colonies from each sample.

Formulation and Synthesis of Zinc Ferrite Nanoparticles

Nanoprecipitation is a simple way to make NPs with a narrow particle size distribution. The following chemicals were purchased: zinc chloride ($ZnCl_2$), iron chloride hexahydrate ($FeCl_3.6H_2O$), sodium hydroxide (NaOH), and oleic acid.

3 Results

The fungi collection and molecular Identification Using ITS Genetic Marker and Phylogenetic Analysis

After making the DNA extraction and visualization on agarose gel electrophoresis after performing PCR for the isolated DNA fungal strains (Figure 1), all fungal strains showed bands in the suspected size. The FASTA sequence produced for each isolate was successfully employed for species identification and the genetic tree was created to validate identification by integrating the new and reference isolates, enabling clear visualization of the established connections.

Subsequently, a phylogenetic tree was done along with the reference isolates into a single species group (Figure 2).

It has been reported that about 12 isolates with 8 different genera, to list as: One was *Aspergillus niger*, one was *A. flavus*, three *Fusarium equiseti*, three *Alternaria tenuissima*, two *Alternaria alternata*, one *Phoma macrostoma*, one was *Trichoderma harzianum*.

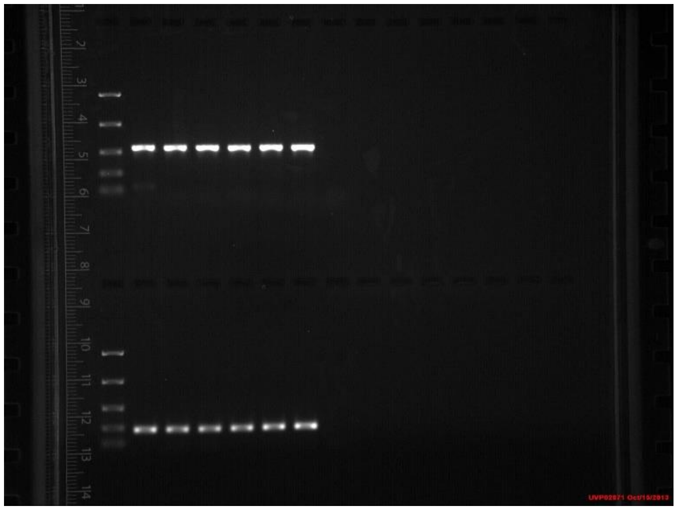

Fig. 1. PCR gel electrophoresis visualization of DNA extracts of fungal strains

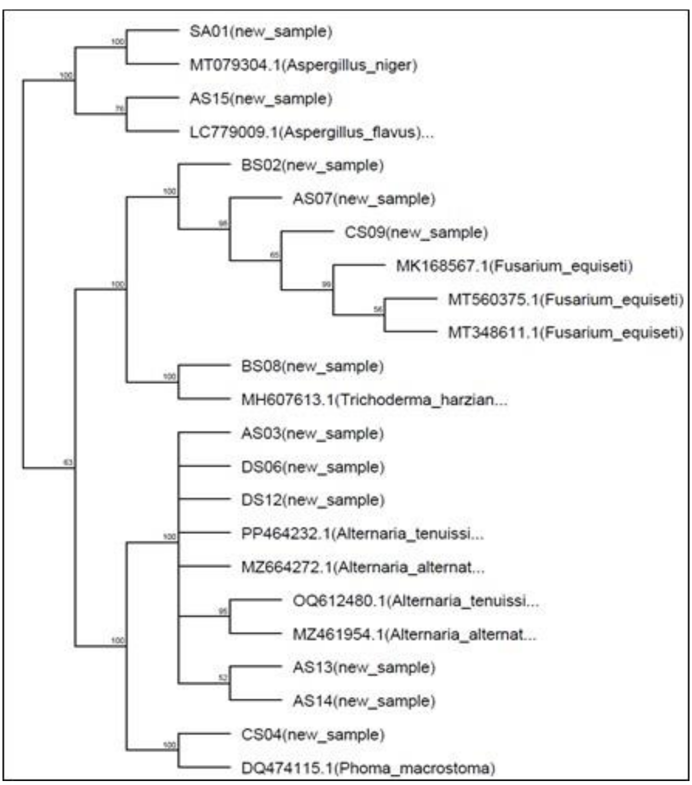

Fig. 2. The phylogenetic tree for the identified fungal strains of 12 fungal strains with different genus and species

Table 1. The olive plant parts, identified strain and codes in this study

N	Isolate code	Species	Host	Plant part
1	AS01	*Aspergillus niger*	Olive	Leaf
2	BS02	*Fusarium equiseti*	Olive	Root
3	AS03	*Alternaria tenuissima*	Olive	Leaf
4	CS04	*Phoma macrostoma*	Olive	Stem
5	DS06	*Alternaria tenuissima*	Olive	Fruit
6	AS07	*Fusarium equiseti*	Olive	Leaf
7	BS08	*Trichoderma harzianum*	Olive	Root
8	CS09	*Fusarium equiseti*	Olive	Stem
9	DS12	*Alternaria alternata*	Olive	Fruit
10	AS13	*Alternaria tenuissima*	Olive	Leaf
11	AS14	*Alternaria alternata*	Olive	Leaf
12	AS15	*Aspergillus flavus*	Olive	Leaf

The previous tables shows that there are main 12 isolates with 8 different genus, to list as : three of *Fusarium equiseti* and *Alternaria tenuissima*, two *Alternaria alternata*, and one of One was *Aspergillus niger, Aspergillus flavus, Phoma macrostoma*, and *Trichoderma harzianum.*

Evaluation of the inhibition capability for plant extract and fungicide against fungal growth.

From different agents used to assess the fungal growth rates in terms of inhibition zones (mm), the high concentration (20 ml) of sesame oil showed significant fungicidal activity with larger inhibition zones (Figures 3, 4, and 5).

Fig. 3. The fungal strains growth and inhibition on PDA medium for CS04

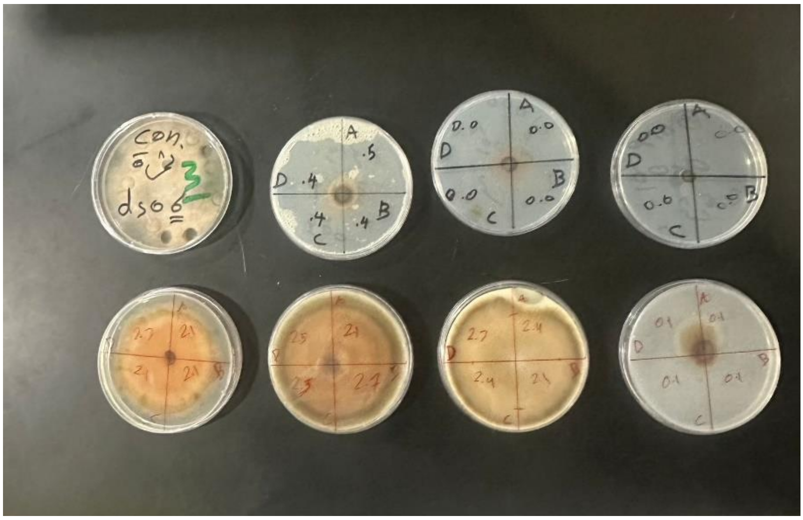

Fig. 4. The fungal strains growth and inhibition on PDA medium for AS03

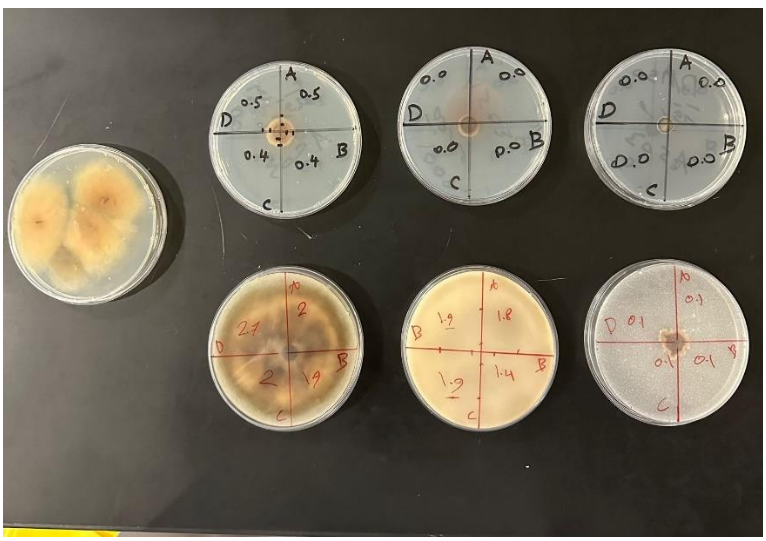

Fig. 5 The fungal strain growth and inhibition on PDA medium for another fungal strain

Every three fungal isolates were represented in a single separate figure as shown. Regarding three fungal isolates named, AS01, BS02, and AS03 represented *as Aspergillus niger, Fusarium equiseti,* and *Alternaria tenuissima* it has been noted that the inhibition zone for Fungicidal agent showed the highest fungicidal activity against BS02 and AS03 with zones (0.291 mm and 0.1 mm), respectively. Sesame oil's two concentrations revealed no large difference in three fungal isolates with inhibition zones (1.63-1.2, 1.35-1.3 and 2.05 - 1.95 mm, respectively). As well as Zinc ferrite nanoparticles optimum concentration was 500 mm with inhibition zones (2.45, 3.37, and 0.45 mm). While plain PDA medium showed fungicidal activity higher than other agents in isolated AS01 and AS03 with inhibition zones (2.28 and 2.46), respectively.

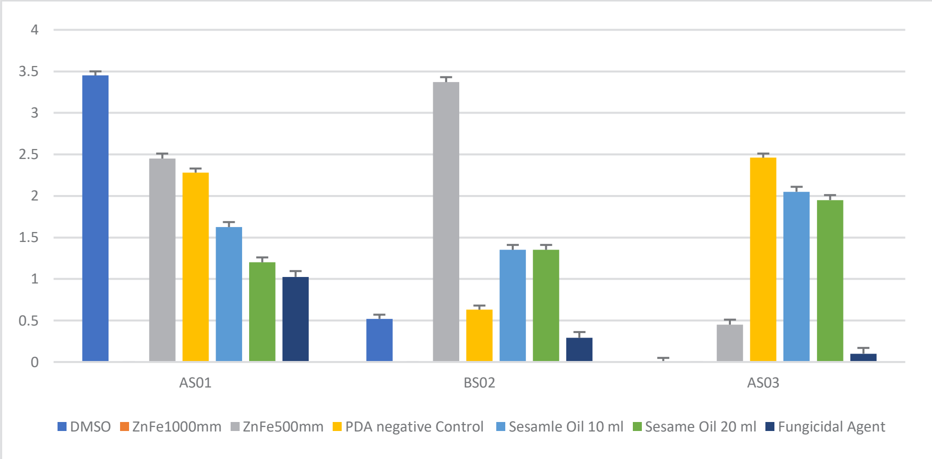

Fig. 6. The evaluation of the inhibition capability for plant extract sesame oil and fungicide against fungal growth for fungal isolates AS01, BS02, and AS03

Other three fungal isolates, CS04, DS05, and DS06, with identified names *Phoma macrostoma*, *Alternaria tenuissima,* and *Fusarium equiseti* report that Sesame oil (20 ml) concentrations showed the highest fungicidal activity with inhibition zones (0.96, 1.31, and 1.90 mm, respectively), either than commercial fungicidal agent which showed only zones of 0.16, 0.99, and 0.15, respectively. PDA plain medium showed no activity in the DS06 isolate and was higher than 10 ml sesame oil in CS04 with an inhibition zone of 0.78 mm.

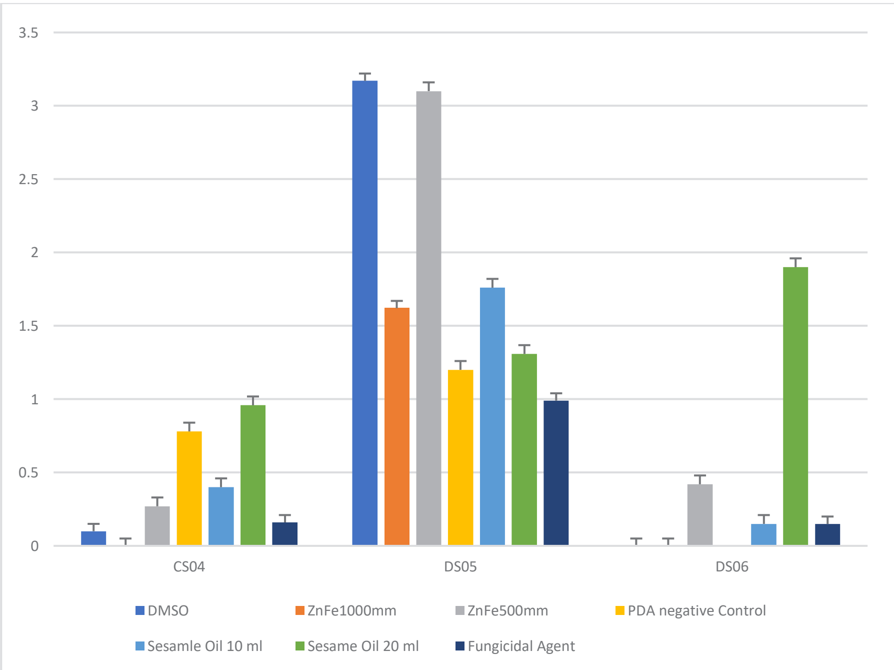

Fig. 7. The evaluation of the inhibition capability for plant extract sesame oil and fungicide against fungal growth for fungal isolates CS04, DS05, and DS06

While other three fungal isolates named AS07, BS08, and CS09 with identified genus and species level as *Trichoderma harzianum, Fusarium equiseti*, and *Alternaria alternata*, to report that AS07 and BS08 showed approximate equal activity of two concentrations of sesame oils with inhibition zones (1.41-1.46 and 4.00 mm), respectively, while fungal isolate CS09 showed highest activity for Sesame oil with concentration 20 ml with inhibition zone 5.84 mm. PDA plain activity against selected fungal isolates showed higher fungicidal activity on three isolates with the highest inhibition activity in BS08 with inhibition zones 2.47, 4, and 2.12 mm, respectively. And 500 mm Zinc ferrite nanoparticles showed a higher activity as fungicide than other concentrations against BS08 and CS09 (2.22 and 1.52 mm, respectively)

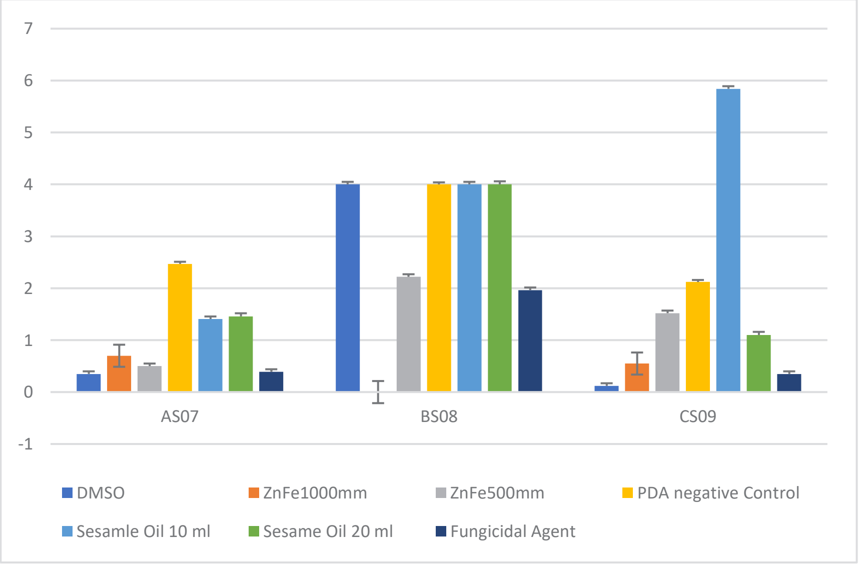

Fig. 8. The evaluation of the inhibition capability for plant extract sesame oil and fungicide against fungal growth for fungal isolates AS07, BS08, and CS09

The following last three fungal isolates, named AS10, DS11, and DS12 with genus level identification *Alternaria tenuissima, Alternaria alternata*, and *Aspergillus flavus*, were tested to reveal that there is no difference between two concentrations of sesame oil for DS12(2.09 and 2.52 mm, for 10m and 20 ml, respectively), while it has activity of only 20 ml concentration for AS10 fungal isolates with inhibition zone 1.74 mm. Zinc ferrite nanoparticles with a concentration of 500 mm showed promising fungicidal activity against DS11 and DS12 with inhibition zones (0.92 and 1.67 mm), respectively.

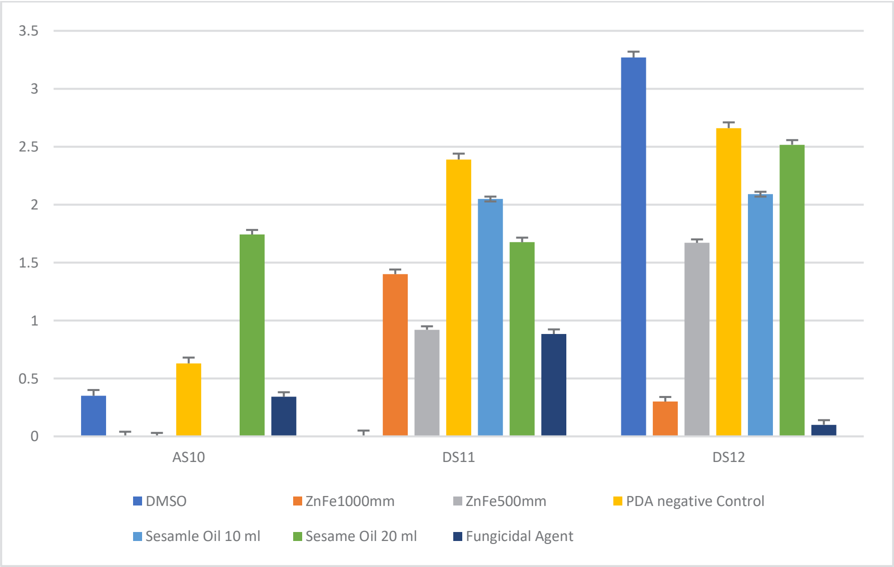

Fig. 9. The evaluation of the inhibition capability for plant extract sesame oil and fungicide against fungal growth for fungal isolates AS10, DS11, and DS12

The fungicidal effect of sesame oil on the highly susceptible fungal strains

The highest fungicidal effect for 10 ml sesame oil was observed against three fungal strains (AS03, DS05, and DS11), while the elevation of the sesame oil concentration raised the fungicidal activity of BS08 to double (Figure 10).

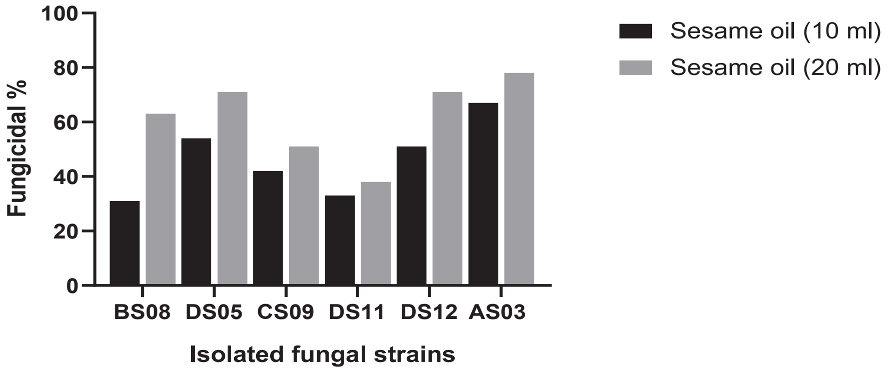

Fig. 10. The fungicidal % of different sesame oil concentrations on highly susceptible isolated fungal strains

4 Discussion

Fungi are significant plant diseases. They are primarily accountable for the majority of plant deterioration that takes place in fields or during storage. This study aims to assess the fungicidal effect of the composited sesame oil against locally isolated fungi in Al Baha and Baljurash regions in Saudi Arabia.

In this study, from different regions of olive cultivation in Al Baha and Baljurashi regions, there is a relatively high percentage of pathogenic fungal infections were observed in the different parts of an olive plant as reported and prevalent of 7 genera classified as Aspergillus niger, Aspergillus flavus, Phoma macrostoma, and Trichoderma harzianum, three of Fusarium equiseti and Alternaria tenuissima, two of Alternaria tenuissima and Alternaria alternata, as reported previously, who found specifically in Al Baha region, from a total of 20 fungal isolates were obtained during this study, and they were classified into five genera and eight species. The identification of these species was accomplished using molecular markers. Aspergillus accounted for 80% of the known species, while the remaining species included B. prieskaensis, F. oxysporum, G. candidum, and Actinomucor elegans and it is in agreement with Almiman (2023b) study, which discovered a total of twenty isolates using molecular methods. These isolates belonged to eight different genera and twelve different species. The predominant species discovered in this study are from the genera Aspergillus (33.3%), Penicillium (16.6%), and Fusarium (16.6%), which are recognized for their ability to produce mycotoxins. As well, it is also inconsistent with the study, which performed an analysis that unveiled 12 fungal species that can be cultivated and have not been previously documented in Arabian desert soils, as well as six more species that have not been recorded in Saudi Arabian desert soils. The genera that were most frequently documented were Aspergillus (found in 20 different locations) and Penicillium (found in 6 different locations). Several commercial fungicides can be used in treating fungal infections that invade plants in all regions and through several mechanisms of action, this study showed moderate activity for commercial fungicide agents against *A niger, Alternaria tenuissima, Fusarium equiseti,* and *Alternaria alternata*, and it is in contrast to Rani et al. (2011) [9], who reported that the commercial fungicide agent was not powerful as the seed extract against *Colletotricum crassipes* which exhibited the largest inhibitory zones (18 mm) compared to other fungi. Subsequently, *Cladosporium* had a diameter of 17.5 mm, followed by Armillaria mellea with a diameter of 17 mm, *Colletotricum capsici* with a diameter of 17 mm, *A.niger* with a diameter of 16.5 mm, and Rhizopus oryzae also with a diameter of 16.5 mm. *A. terreus* and Candida albicans exhibited smaller inhibition zones (15.5 and 16.0 mm) in comparison to the other species.

The sesame oil revealed the highest antifungal activity when tested against several isolated fungal isolates, and it is in agreement with Lavaee et al. (2019) [7], who reported that the minimum concentration required to kill fungus was also assessed. The species that was most found was *C. albicans*, followed by *A. niger* and then *C. glabrata*. The findings revealed that all *C. albicans*, *C. glabrata*, and *Phoma macrostoma* isolates showed sensitivity to antifungal drugs but resistance to 20 ml of sesame extract and sesame oil. In addition to a study conducted by Syed et al. (2015) [10], who reported that the metabolites were obtained from the leaves, stems, and roots and evaluated for their activity against the plant pathogenic fungus *Macrophomina phaseolina, Alternaria sesami*, and *Fusarium oxysporum*.

It has been reported that Zinc ferrite nanoparticles are promising form as antifungal agents in plants, this study showed a high activity of only 500 mm of zinc ferrite nanoparticles against several genera of fungi such as *Aspergillus niger, Fusarium equiseti*, and agrees with Naik et al. (2019) [11], who reported that tZnFe2O4 nanoparticles exhibited potent antifungal properties against various plant fungal diseases, including Aspergillus niger and *Fusarium equiseti* [12-14].

5 Conclusion

Olive plants in the regions of Al Baha and Baljurashi in Saudi Arabia are infected with several types of fungi such as *Aspergillus niger, Fusarium equiseti, Alternaria tenuissima, Phoma macrostoma,* and others, plant extract such as sesame oil with maximum concentration can reveal strong fungicidal activity when compared to commercial fungicides and Zinc ferrite nanoparticles against several fungal isolates specifically, *Aspergillus niger,* as well, Zinc ferrite with concentration 500 ml revealed a promising antifungal activity when compared to higher concentration against *Alternaria tenuissima*, and only 10 ml sesame oil is enough to combat fungal infections for several fungal isolates in olive plants such as *Trichoderma harzianum, Fusarium equiseti,* and *Alternaria alternata*. Therefore, several antifungal approaches must be implemented to combat fungal infections in Al Baha region and other nanoparticles with silver nitrate and PEG inserted must be involved in many clinical trials to be used as plain antifungal agents.

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