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ORIGINAL ARTICLE

THE EFFECT OF SOME ALCOHOLIC EXTRACTS OF SOME PLANTS IN REDUCING THE INFECTION OF FUNGI ASSOCIATED WITH PLANT TISSUE CULTURE

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Abstract: The study was conducted in the Date Palm Research Center, University of Basrah, 2018-2019. The study results showed the isolation of three types of fungi associated with in vitro culture of plant tissues (*Alternaria alternata*, *Chaetomium globosum* and *Fusarium solani*). Two types of plant extracts were also used, the alcoholic extract of pomegranate peels. And the black seed in three concentrations (0, 5, 10 and 15%). The results showed a close effect of the extract type on the percentage of inhibition of fungi isolated from tissue culture. The type of extract led to the inhibition of the fungus *A.alternata* with an inhibition rate of 46.80% and 45.83% for the extracts of pomegranate peels and black seed on the type of extract also affected the percentage of *C. globosum* inhibition with an inhibition rate of 42.22% and 41.39% for the extracts of pomegranate peel and black seed, respectively. The extract of pomegranate peels and black seed recorded an inhibition rate of *F. solani* that amounted to 48.61% and 48.33%, respectively. The study results showed the effect of different concentrations of the extracts used in the study (pomegranate peels and black seeds) on the inhibition of the growth of isolated fungi in varying proportions. According to the concentration used, the highest rate of inhibition of fungi isolated from plant tissue culture *A. solani* when treated with the concentration of 15% and it was 73.33% 73.88% 75.55% for the three fungi respectively, with significant differences from the concentrations 0%, 5% and 10%. The highest rate of inhibition of the growth of isolates of fungi *A. alternata*, *C.globosum* and *F. solani* was recorded at 15% concentration for pomegranate peel and black seed extracts, which reached 74.99% and 71.66% (75.00% and 72.78%) 76.11% and 75.00%), respectively, with significant differences from the other concentrations used in the study.

***Key words*:** Plant extracts, Tissue culture, Fungi, Date palm.

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1. Introduction

Date palm tree, *Phoenix dactylifera* L., belongs to the palm family Arecaceae; it is one of the most important fruit trees widely cultivated in Iraq and some regions of the Middle East [Matar (1991)]. Despite the availability of suitable conditions for the cultivation and production of date palms in the countries of the Arab world, due to the appropriate climate and soil, the production of date palm is low compared to other countries of the world. One of the reasons for this is poor performance in agricultural operations, reliance on traditional methods, inefficient use of agricultural resources and exposure of date palms to many pests and diseases that limit date palm production in the Arab world.

Medicinal plants occupy a great place in agricultural and industrial production and are considered the primary source of active substances. Pomegranate is one of the fruits of *Punica granatum* L. tree. In recent years, the number of published researches on this fruit has doubled due to its great importance and role in human nutrition and health, in addition to containing many Effective compounds, the most important of which are tannins, alkaloids, anthocyanins, terpenes, flavonoids,

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soaps, resins and others that act as anti-bacterial, viruses and fungi. Phenolic compounds are generally characterized by their ability to scavenge free radicals, bind metal ions and stimulate antioxidants, thus breaking the chain of oxidation reactions. Thus, they participate in the first line of defence against free radicals [Al-Hasany *et al*. (2020)]. Infection with fungi at high rates and better than chemical fungicides [Daniel *et al*. (2015)], such as aqueous extracts of pomegranate peels, inhibited the growth of *Alternaria alternata* isolated from apple fruits with a 75% inhibition [Obeid *et al*. (2013)]. The effectiveness of the extracts of the same plant may vary according to the different solvent, the use of the extract or according to the different plant part used and also according to the age stage of the plant part used itself, or the effectiveness of plant extracts may differ according to the phase of the plant treated with the extract [Noaema *et al*. (2020a)]. Fungi are one of the most important pathogens that cause great losses in agricultural production. Chemical control of fungi is still the most successful method in controlling fungal diseases of plants compared to the other methods used in control. However, the indiscriminate use of these chemicals has increased resistance. The fungi are in plants, so natural products have been searched for as an alternative to these synthetic fungicides because they are not harmful to health and contribute to environmental protection.

The black seed plant (*Nigella sativa*) is a herbaceous plant with threadlike leaves and white star-shaped flowers with a distinctive aromatic smell. It contains Nigellon, a crystalline substance extracted from the seeds, which is the natural active substance found in the plant and is considered one of the effective antioxidants against free radicals. Its seeds are black, with an aromatic smell and taste, and contain more than 30% fixed oils, 4% volatile oils and 20% protein. The misuse or overuse of antibiotics has led to strains of bacteria and fungi working to develop their ability to produce substances that disrupt the action of antibiotics or change their target or ability to penetrate the cells of these microbes [Noaema *et al*. (2020b)]. They are also rich in some mineral salts and vitamins. It contains many essential fatty acids and is one of the antioxidants, and the extracts of this plant have been used to inhibit many pathogenic microorganisms in humans and animals due to the presence of Thymol and Thymoquinone and the black seed contain phenolic compounds that have a direct effect in inhibiting microorganisms. Several studies indicated the efficacy of these natural plant extracts and their toxicity for many pathogens [Obeid *et al*. (2013), Jiménez-Reyes *et al*. (2019)]. With this increase in the strains resistant to chemically manufactured antibiotics, humans have resorted to finding suitable alternatives to these compounds and using plant-based antibiotics that are safer and less harmful than their chemical counterparts. Therefore, the effectiveness of some plant extracts related to pomegranate peels and a black seed was tested to see how effective they are against associated fungi for tissue culture as natural alternatives to chemicals.

2. Materials and Methods

2.1 Isolation of fungi associated with tissue culture

The associated fungi were isolated after the appearance of symptoms of fungal contamination of the tissues, after washing the plant pieces with sterile distilled water to remove traces of the culture medium used for tissue culture for several times and then dried with sterile filter paper. Then, the pieces were transferred to the sterile culture medium for isolating fungi (PDA) in a steam sterilization device. The antibiotic Chloramphenicol was added at a concentration of 250 $mg.l^{-1}$. The dishes were incubated in the incubator at a temperature of 25±2ºC for 3 days and then examined, the fungi isolated from tissue culture were purified and identified based on Ellis (1976), Barnett and Hunter (1972) and Domach *et al*. (1980) were isolated 3 associated fungi from tissues of plants are *Alternaria alternata*, *Chaetomium globosum* and *Fusarium solani*.

2.2 Preparation of alcoholic extracts

The extracts were prepared according to the Harbone (1973) method in preparing plant extracts, with a weight of 100 gm of black seed powder and pomegranate peels, each separately, 500 ml of ethanol alcohol was added at a concentration of 98%, mixed well and left for 24 hours at laboratory temperature (25°C), after which the extract was filtered. Using filter paper (Whatman No 1), the filtrate was concentrated in a rotary evaporator at 40°C to remove the solvent and left to dry in a petri dish at laboratory temperature to completely remove the solvent until a highly viscous concentrate was obtained. This material is 50 ml of distilled water to make the concentrations used in the study.

2.3 Chemical detection of some active substances in the studied plant extracts

pH estimation: Put 5 g of dried powder in 50 ml of distilled water of pH 7 Mix the mixture in a magnetic stirrer for 10 minutes. The mixture was filtered, and the pH values were measured using a pH meter.

Detection of saponins: Saponins were detected in the plants used in the experiment. A tight-sealed bottle tower containing the aqueous extract and the appearance of thick foam that remains for a long time on the surface of the extract is a positive result of the detection [Harborne (1984)].

Detection of holding materials Tannins: The adopted method of Harborne (1984) was used as follows:

Take 5 grams of the vegetable powder, add to 25 ml of 95% ethyl alcohol, leave for one minute in a water bath at a temperature of 100°C, filter the solution, and add to it 100 ml of hydrochloric acid.

Detection of phenols: A 1% aqueous solution of ferric chloride was added to an equal amount of the aqueous extract; the presence of phenols was indicated by the appearance of a bluish-green precipitate [Harborne (1984)].

2.4 Studying the effect of different concentrations of studied plant extracts on inhibiting the growth of fungi isolated from tissue culture

The effectiveness of concentrations (5, 10, 15%) of the studied extracts (pomegranate peel and black seed) was evaluated. Each concentration of extracts was transferred separately and mixed with 250 ml of sterilized and previously cooled PDA medium. Pour the culture medium containing the required concentration for each extract into sterile glass dishes with a diameter of 9 cm. Inoculate the center of the dish with a 0.5 cm diameter disc of isolating each fungus isolated separately growing on PDA culture media at the age of 7 days using a sterile cork piercing, with 4 replicates with the application of a comparison treatment by inoculation the center of the dish is a disk with a diameter of 0.5 cm from isolates of fungi isolated from tissue culture only, both individually grown on PDA culture media and without any extract. The dishes were incubated at a temperature of 25 ± 2ºC and the radial growth of the fungi isolated from the tissue culture was measured by taking the average of two perpendicular diameters passing from the bottom of the plate and after the fungus growth reached the edge of the plate in the control treatment. For each factor of the experiment and the interaction between them according to Abbot's (1925) equation.

$$\text{Percentage of inhibition} = \frac{\text{The radial growth rate in comparison} - \text{The radial growth rate in the treatment}}{\text{The radial growth rate in comparison}} \times 100$$

2.5 Statistical analysis

The factorial experiments were carried out according to the Completely randomized design (C.R.D) with two-factor experiments (fungi isolated from tissue culture and studied extracts with different concentration), the averages were compared according to the least significant difference method, the average L.S.D under the level of significance 0.01.

3. Results

3.1 Chemical detection of some active compounds in the studied plant extracts

The chemical detection of pomegranate peels and seeds black seed gave a positive result for saponin, tannins and phenols (Table 1). The results indicate the active role of the alcoholic extracts of pomegranate peels and black seed in inhibiting the growth of fungi and the importance of the active compounds in those extracts. Hamim (2003) mentioned that the efficiency of alcohol in extracting the active compounds is more than water. In general, the effectiveness of these extracts may be due to their containing most of the active compounds and the anti-fungal activity of these compounds. Tylor *et al*. (1996) indicated that these phenolic and tannin compounds have anti-fungal activity. It is characterized by its ability to unite with the cell protein and precipitate it, changing its nature and working as a suitable solvent for fatty substances; that

Table 1: Chemical detection of the presence of some active compounds in the peels of pomegranate fruits and black seeds.

No.	Active compounds	Pomegranate peels	Black seed
		Alcoholic extract	
1	Saponins	+	+
2	Tannins	+	+
3	Phenols	+	+
4	Acid function	6.5	6

(-) Detection is negative, (+) Detection is positive.

Table 2: The effect of the extract type in inhibiting the growth of fungi isolated from culture of date palm tissues *in vitro*.

Extracts	*A. alternata*		*C. globosum*		*F. solani*	
	Fungus average diameter (cm)	Inhibition ratio (%)	Fungus average diameter (cm)	Inhibition ratio (%)	Fungus average diameter (cm)	Inhibition ratio (%)
Pomegranate peels	4.87	46.80	5.21	42.22	4.62	48.61
Black seed	4.78	45.83	5.27	41.39	4.73	48.33
L.S.D.	0.170	1.89	0.095	1.108	0.271	1.108

Table 3: The effect of concentration in inhibiting the growth of fungi isolated from culture of date palm tissues *in vitro*.

Concentration (%)	*A. alternata*		*C. globosum*		*F. solani*	
	Fungus average diameter (cm)	Inhibition ratio (%)	Fungus average diameter (cm)	Inhibition ratio (%)	Fungus average diameter (cm)	Inhibition ratio (%)
0	9.00	0.00	9.00	0.00	9.00	0.00
5	4.62	48.61	5.25	41.66	4.40	53.05
10	3.30	63.33	4.35	51.66	3.12	65.27
15	2.40	73.33	2.37	73.88	2.20	75.55
L.S.D.	0.241	2.680	0.135	1.567	0.384	1.567

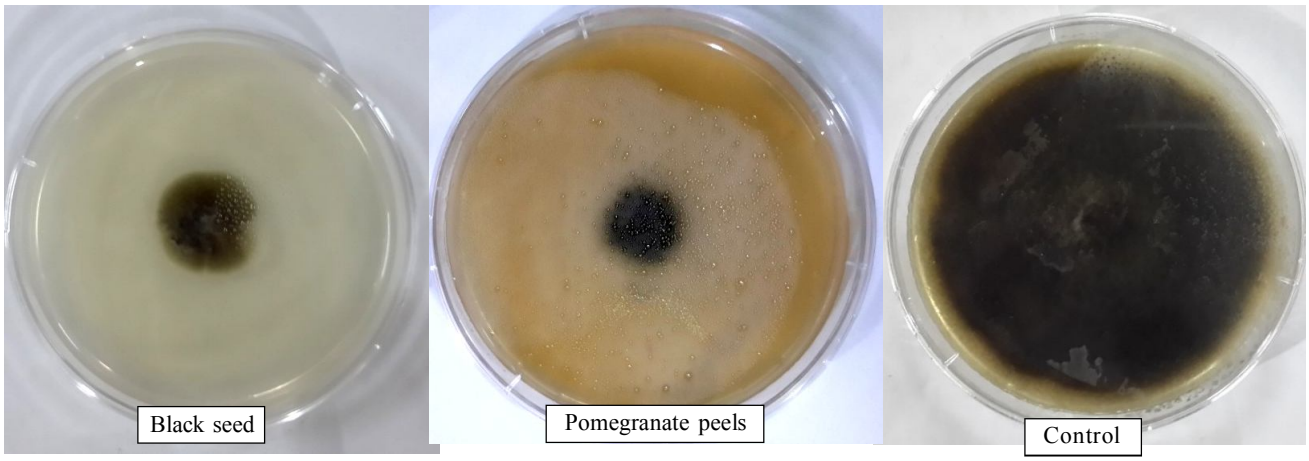
is, it decomposes the membranes of living cells, and as a result, the inner cellular components come out, so the fungal and bacterial cell dies and the positive chemical detection of phenols, saponins and tannins enhance this importance against fungi.

3.2 Effect of the extract type in inhibiting the growth of fungi *A. alternata*, *C. globosum* and *F. solani*

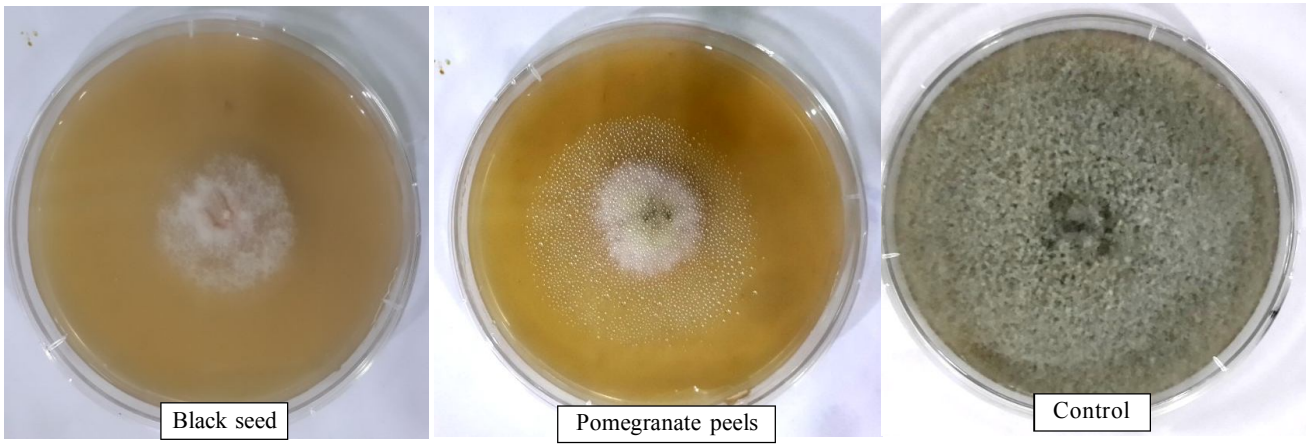
The effect of the extract type in inhibiting the growth of fungi isolated from tissue culture *A. alternata*, *C. globosum* and *F. solani* was studied. The results showed a close effect of the extract type on the percentage of inhibition of the fungi, as mentioned earlier. Table 2 shows the effect of the extract type on the percentage of inhibition. The fungus *A. alternata* and pomegranate peel extract's inhibition percentage and black seed extract seeds were 46.80% and 45.83%, respectively. The same table indicates that the inhibition average of *C. globosum* for pomegranate peel and black seed extract was 42.22% and 41.39%, respectively. While the percentage of inhibition of *F. solani* for pomegranate peel extract and seeds black seed was 48.61% and 48.33%, respectively.

3.3 Effect of different concentrations of studied plant extracts (pomegranate peel and seeds black seed) on inhibiting the growth of fungi *A. alternata*, *C. globosum* and *F. solani*

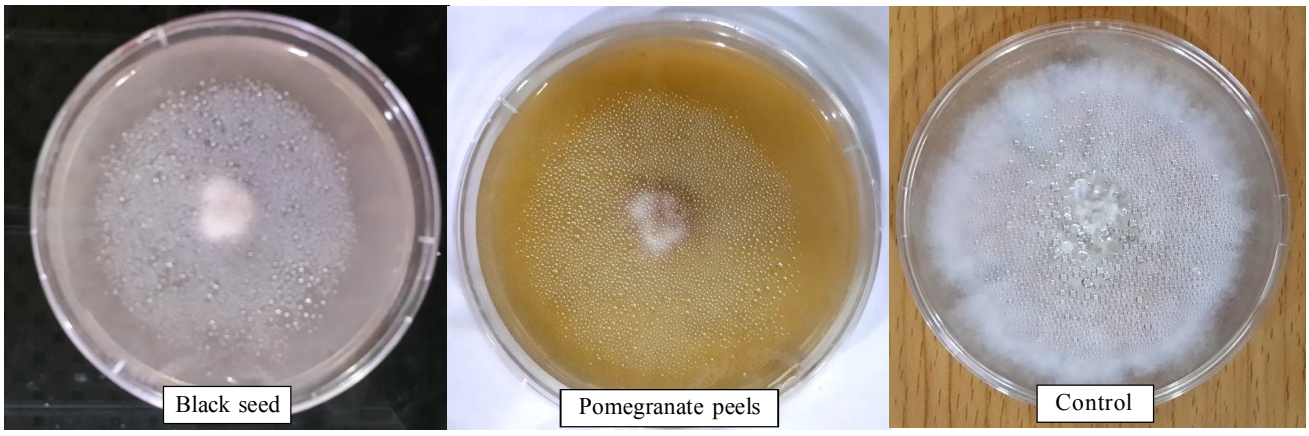
The results showed the effect of different concentrations of the extracts used in the study (pomegranate peels and seeds black seed) on the inhibitory activity of the isolated fungi in varying proportions and according to the concentration used 0%, 5% and 10%, which amounted to 0.00%, 48.61 and 63.33%, respectively (Table 3, Fig. 1). The results showed the effect of the different concentrations of the two extracts used in the study (pomegranate peel and seed black seed) on the inhibitory activity of the isolated fungi in varying proportions and according to the concentration used. The highest inhibition rate of *C. globosum* was recorded, when treated with 15% concentration, which was 73.88%, with significant differences from 10%, 5% and 0% concentrations, which amounted to 51.66%, 41.66% and 0.00%, respectively. The results showed the effect of different concentrations of the extracts used in the study (pomegranate peels and seeds black seed) on the inhibition of the growth of isolated fungi in varying proportions and according to the concentration used. The highest inhibition rate of *F. solani* was recorded when treated with a concentration of 15%; it was



Effect of the interaction between extract type and different concentrations of studied plant extracts (pomegranate peels and seeds black seed) in inhibiting the growth of *A. alternata*.



Effect of the interaction between extract type and different concentrations of studied plant extracts (pomegranate peels and seeds black seed) in inhibiting the growth of *C. globosum*.



Effect of the interaction between extract type and different concentrations of studied plant extracts (pomegranate peels and seeds black seed) in inhibiting the growth of *F. solani*.

Fig. 1: Effect of the interaction between extract type and different concentrations of studied plant extracts (pomegranate peels and seeds black seed) in inhibiting the growth of fungal growth *A. alternata*, *C. globosum* and *F. solani*

Table 4: The effect of interaction in inhibiting the growth of fungi isolated from culture of date palm tissues *in vitro*.

Extract type	Concentration (%)	*A. alternata*		*C. globosum*		*F. solani*	
		Fungus average diameter (cm)	Inhibition ratio (%)	Fungus average diameter (cm)	Inhibition ratio (%)	Fungus average diameter (cm)	Inhibition ratio (%)
Pomegranate	0	9.00	0.00	9.00	0.00	9.00	0.00
	5	4.60	48.88	5.30	41.11	4.25	52.78
	10	3.30	63.33	4.25	52.77	3.10	65.55
	15	2.25	74.99	2.30	75.00	2.15	76.11
Black seed	0	9.00	0.00	9.00	0.00	9.00	0.00
	5	4.65	48.33	5.20	42.22	4.55	53.33
	10	3.30	63.33	4.45	50.55	3.15	64.99
	15	2.55	71.66	2.45	72.78	2.25	75.00
L.S.D.		**0.341**	**3.791**	**0.191**	**2.217**	**0.543**	**2.217**

75.55% with significant differences from 10% concentrations, 5% and 0%, which amounted to 65.27%, 53.05% and 0.00%, respectively (Table 3).

3.4 The effect of the interaction between the type of extract and the different concentrations of the studied plant extracts (pomegranate peel and seed black seed) on the inhibition of the growth of the fungi *A. alternata*, *C. globosum* and *F. solani*

The results of the experiment in Table 4 shows that the alcoholic extract of pomegranate peel and black seed recorded the highest average of inhibition of the growth of the fungus *A. alternata* at the 15% concentration, reaching 74.99 and 71.66%, respectively, with significant differences from the other concentrations used in the study. The percentage of inhibition recorded the growth of the fungus at concentrations of 10%, 5% and 0% reached 63.33%, 48.88% and 0.00% for pomegranate peel extract and 63.33%, 48.33% and 0.00% for black seed extract, respectively (Table 4). The results of the experiment showed that high concentrations of pomegranate peel extract inhibited the growth. The highest percentage of growth inhibition was recorded in *C. globosum* fungus at 15% concentration for pomegranate peel extract and seeds black seed, which amounted to 75.00% and 72.78%, respectively, with significant differences from the concentrations. Used in the study, the inhibition of fungal growth was recorded at 10%, 5%, 0%, 52.77%, 41.11%, and 0.00% for pomegranate peel extract, and 50.55%, 42.22% and 0.00% for black seed extract, respectively (Table 4).

Table 4 indicates the effect of both extracts and the different concentrations used in the study and at averages that varied between them significantly and according to the extract type and concentration. The highest inhibition of the growth of the fungus *F. solani* was recorded at the 15% concentration for the extract of pomegranate peels and seeds black seed, which amounted to 76.11% and 75 .00%, respectively. As for the low concentrations, they were insufficient to achieve the desired effect. This may be due to the low concentration of the active substances in these concentrations. The inhibition rate for the fungus growth was recorded at 10%, 5% and 0%, 65.55%, 52.78% and 0.00% for the extract of peels of Pomegranate 64.99%, 53.33% and 0.00% of black seed extract, respectively.

4. Discussion

The effectiveness of plant extracts (extract of pomegranate peel and seeds black seed) in inhibiting fungi isolated from tissue culture may be because these plants contain chemical compounds that have biological and biological activity against microorganisms, which were liberated when added to the culture medium, which led to a change in the natural properties of the medium and made it a medium Less suitable for the growth of pathogenic fungi, and the reason for the inhibition may be due to the content of the extracted materials on some compounds such as phenols, saponins and tannins, and

these compounds have anti-fungal activity. The inhibition mechanism of these compounds have been explained to direct contact with microorganisms and the destruction of the plasma membrane, and that the studied plants contain those chemical compounds that may be attributed to the inhibitory activity, as these compounds can penetrate the cell membrane and block the active sites of some enzymes, which may be necessary for the growth and reproduction of the organism inside the fungal cell and thus lead to inhibition of the growth of pathogenic fungi or due to the interaction of these compounds in the series of metabolic reactions necessary for growth and reproduction [Tylor *et al*. (1996) and Anthony (1976)]. Sarah (2015) stated that pomegranate peels are rich in secondary metabolites represented by alkaloids, saponins, sterols, triterpenes, glycosides and tannins. Guo *et al*. (2009) extracted a peptide antifungal named Pomegranin that can inhibit mycelium growth of *Fusaruim oxysporium* and *Botrytis cinerea*.

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