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Original Research Article

Using Plant Extracts and Fungus Antagonisms Against Corn Leaf Spot Disease Caused *curvularia clavata*

Hassan A. Tamur[1*]
[1]Applied Biotechnology Department, College of Biotechnology, Al-Qasim Green University, Babylon 51013, Iraq

***Corresponding Author:** Hassan A. Tamur
Applied Biotechnology Department, College of Biotechnology, Al-Qasim Green University, Babylon 51013, Iraq

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Abstract: The inhibitory activity of the flour substance attached to the growing tip of the palm tree and the pomegranate peel substance was tested on the spores of the fungus *Curvularia clavata*. The results showed a high percentage of the effect of the two substances on the fungus spores. A concentration of 1 g/L gave a higher effect on the fungus spores, while pomegranate peels gave a concentration of 10 g/L. The effect of both was significant, especially at the two mentioned concentrations compared to the control treatment, as the yellow substance treatment gave a greater effect when compared to the pomegranate peel treatment. Time also affected the spores of the fungus, especially on the seventh day. In addition, it was noted through the results that there were significant differences in the interaction of time with the treatments, especially in the yellow substance treatment and the pomegranate peel treatment compared to the control. The same is the case when the interaction of time with the concentrations gave a significant effect, especially at the concentration of 10 g/L. The interaction of the treatments with the concentrations gave great results in the effect between the treatments and concentrations, especially the yellow substance treatment, as well as the Pomegranate peels when compared to the control, in addition to the interaction between the experimental factors had a significant effect on the bactericidal effect of C.clavata compared to the control treatment.

Keywords: *Curvularia clavata*, *Trichoderma orientale*, *Marasiemllus palmivorus*, yellow powder, pomegranate peel, corn.

1- INTRODUCTION

c. clavata (Jain) is a fungus belonging to the Dematiaceae family Fam. Pleosporaceae, Ord. Pleosporales, Cla. Loculoascomycetes, Phy. Ascomycota, which produces a fungal yarn color and fungal structures that are brown, other brown, from a diverse group of thallus, as it has many genera, some of which are found in the soil and on plant remains. The fungus is widespread in tropical areas (Thomas *et al.*, 1988). Many of them cause diseases in humans and animals, such as severe allergies in the respiratory system and pneumonia, in addition to the side effects caused by the fungus, such as skin diseases caused by some of its types, as some fungi carry toxins from the spores due to metabolism (Geiser *et al.*, 2000; Miller, 1998). It also causes severe itching in sheep and goats (Thanaa *et al.*, 2008). It is one of the most important fungi that infect humans. Wounds are a very helpful factor for infection with this fungus. It causes many skin infections and results in severe itching, especially in areas, In areas where hair is abundant and areas that are not directly exposed to sunlight, such as the chest, abdomen, and lower jaw, severe infection, if left untreated, leads to swelling, which results in hair loss in the affected areas due to severe itching, and thus swelling of these areas, in addition to the spread of the infection to all parts of the body in severe cases. The infection also results in peeling of the skin and appears as if it were in the form of blisters. The infection also appears on the scalp, especially in the back of the head, also in the form of blisters. In some cases, a yellow liquid comes out of these blisters when scratched hard, causing swelling. It was also mentioned that it affects the nervous system, in addition to wound inflammation, and sometimes causes weakness, tumors, and pus when eating food contaminated with it. Its danger can range from moderate to fatal (Fan *et al.*, 2009 and Hiromoto *et al.*, 2008). The fungus also causes chronic sinusitis, which causes severe pain in the forehead and also causes headaches, in addition to causing lethargy and drowsiness and causing secretions that are often yellow in color. In severe cases, it causes a tumor in the skull bones and general emaciation. It can be treated by using some antifungal agents designated for this purpose (deShazo *et al.*, 1997). Some types of the fungus *C. clavata* cause diseases on plants such as spots and others on plants.

Some fungicides have been used to reduce its damage, in addition to using isolates of Bacillus spp. against the fungus *C. lunata*, which causes wilting on corn plants (Chandrashekar *et al.,* 2000). It produces conidia similar to the fungus Alternaria spp., except that they are curved and the fungus forms a pad on which the conidia rest. This fungus is one of the well-known and dangerous fungi for humans and animals because it causes severe skin infections that may lead to symptoms similar to scabies when the infection is severe and its symptoms are severe itching. Then the skin infection site peels. Moderate environmental conditions are suitable for the fungus *Curvularia spp*. Initially, it was known to infect plants, as it was first discovered and described as causing corn leaf spots by (Mandokhod and Basu Choudhary 1972). Some of its genera were recently discovered to infect humans and animals. It is transmitted by spores that are carried by the wind. The spores are curve-shaped, hence the name of the genus. The fungus resembles the fungus Alternaria spp., except that the spores of the fungus *Curvularia spp* are characterized by swelling from the middle area or from the corner from which the name came. The research aimed to find a safe and effective way to reduce its harm to humans and animals.

2 -MATERIALS AND METHODS

2-1-Propagation of inoculum isolates of *curvularia clavata, Trichoderma orientale* & *Marasiemllus palmivorus*.

The fungal inoculum was prepared using panicum malaceum seeds by soaking them in water for a period and washing them well to remove dust and impurities, then placing 50 gm of them in a 500 ml flask, plugging its mouth with sterile cotton and sterilizing it in an autoclave at 121℃ and 15 lb/in2 pressure for one hour, then the sterilization process was repeated the next day under the same temperature, pressure and time mentioned. Each petri dish was inoculated with five discs, each 0.5 cm in diameter, from the growing medium on which the fungus was incubated at 25 ± 2 °C for 3 days,

2-2- Antifungal Index Evaluation

This study was conducted by using the Dual culture plate test method on the PDA medium under sterile conditions. growths of the tips of the hyphae of both the antagonist and the pathogenic fungus were placed opposite each other on the edges of the dish simultaneously. Disks of the pathogenic fungus only were placed in the dishes for all treatments as a control. Each treatment contained three (3) replicates. The dishes were incubated at a temperature of 25 ± 2 °C. The growths of the pathogenic fungus were recorded from the beginning of its formation until a period of 5 m, coinciding with the completion of the growth of the colonies of the antagonist fungus to the edges of the control dishes (Asad, *et al.*, 2014).
Inhibition rate %: (colony diameter growth in control-treated colony diameter growth/colony diameter growth in control)* 100.

2 -2-1- Extract preparation.

A floury substance was collected that was attached to the growing apex leaves of the palm tree, which had a yellowish brown color and resembled animal manure when ground and crushed by hand, and the second substance was pomegranate peels. The hot aqueous extract was prepared according to the method of (Rios *et al.*, 1987) by mixing 40 g of each substance with 160 ml of distilled water, i.e. at a ratio of (1: 4) g/volume after crushing the two samples with a mortar, then the mashing was completed using an electric blender (Blender) and the mixture was left in the refrigerator for 24 hours for soaking. The mixture was filtered through several layers of gauze and filtered again using a Buechner funnel using filter paper by centrifuging the extract. The clear substance was placed in glass bottles with a capacity of (50) ml and kept in the refrigerator, then placed in the dryer (Lyophilizer). Under the vacuum pressure provided by (Edward High Vacuu V.K.) to dry them at a temperature of (-50 m5), the samples were kept in tightly sealed glass containers until use. After that, 1 gram of the raw plant extracts was taken and dissolved in (5) ml of sterile water. This resulted in obtaining an extract with a concentration of (200) mm/ml as a standard concentration. After that, a (0.22) micron diameter Seitz filter was used. After preparing the extracts and sterilizing them, it was placed in a water bath at a temperature of 100 C (Al-Naaman, 1998). After that, limited weights of the extracts were added to limited volumes of the culture medium (PDA) before solidifying in glass bottles with a capacity of (250) ml after shaking them well so that the extract is homogeneous with the culture medium. We obtained a concentration of (1, 5, 10) g/L. According to the equation N1V1 = N2V2, it was poured into five Petri dishes with a diameter of (9) cm. After the media solidified, a disk was taken from the edge of the fungal colony at the age of one week using a cork piercer with a diameter of 0.5 cm. The disk was placed in the center of the dish under sterile conditions. Then the dishes were incubated at a temperature of (27 -+ 2) in an incubator (Memert Germany) for 5 days. Then the results were taken for the fungus germination and in multiple stages, i.e. on days 7, 9, 11. It was based on three replicates for each concentration (Pitt and Hocking, 1997). The yellow substance extract was symbolized by the symbol D and the pomegranate peels by the symbol P).

2 -2 – 2- Calculation of sporulation.

Three discs were taken from the colony of *C. clavata* growing on PDA medium in Petri dishes using a cork piercer and the discs were placed in 10 ml plastic bottles containing 5 ml of the fixed solution consisting of Alcohol, Formalin, and Glacial Acetic acid in a ratio of (8:1:1) volume to volume and were stirred gently for a few seconds to separate the mature conidia from the conidiophores that were to be counted. Then the numbers of conidia formed were counted using a Haemocytometer and the conidia stuck together as well as the deformed conidia were excluded. Three readings were taken for each bottle (Bashi and Aust, 1986). One drop of the fixed solution containing the conidia was placed and covered

with a slide cover and calculated at a power of 10× with three replicates for each treatment and three readings for each replicate. The numbers of conidia were estimated on the 7th, 9th, and 11th day (Ghali and Al-Janabi, 1995). The germination rate was calculated according to the following equation:

$$\text{Number of conidia} = \frac{\text{Reading rate} * \text{volume of fixed}}{\text{Hemocytometer volume}} \text{ -- The result is divided by the disk distance}$$

Abood and Losel, (1991).

2-3- Statistical analysis

The experiments were designed using a completely randomized design (CRD), and the data obtained were analyzed by analysis of variance using the Statistical Analysis System (SAS) program. Duncan test was used to compare the means at a significance level of ($P > 0.05$).

3- RESULTS

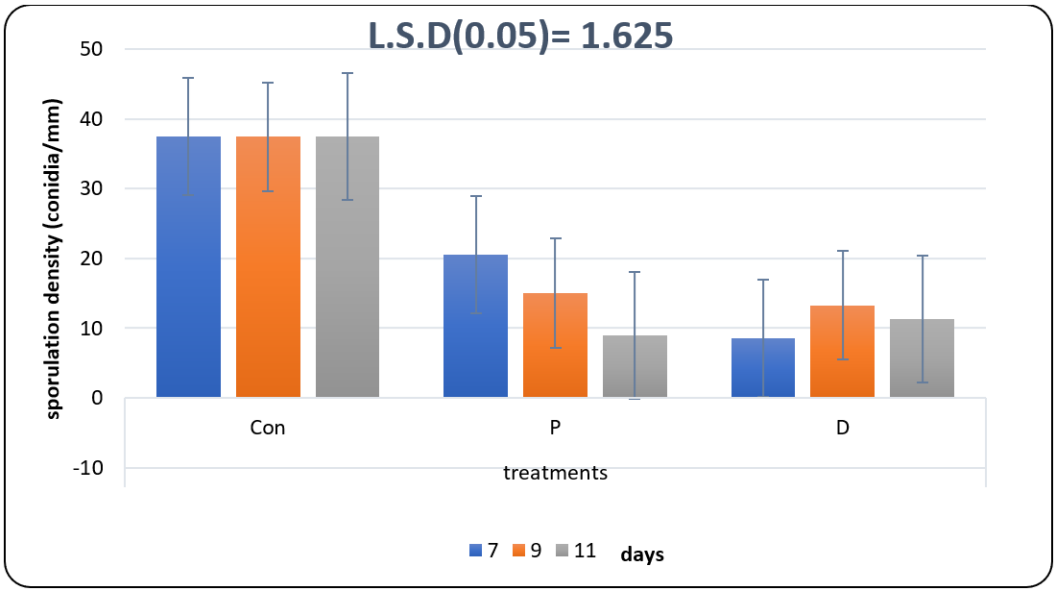

Fig. 1: Effect of time interaction with treatments on *C.clavata* sporulation

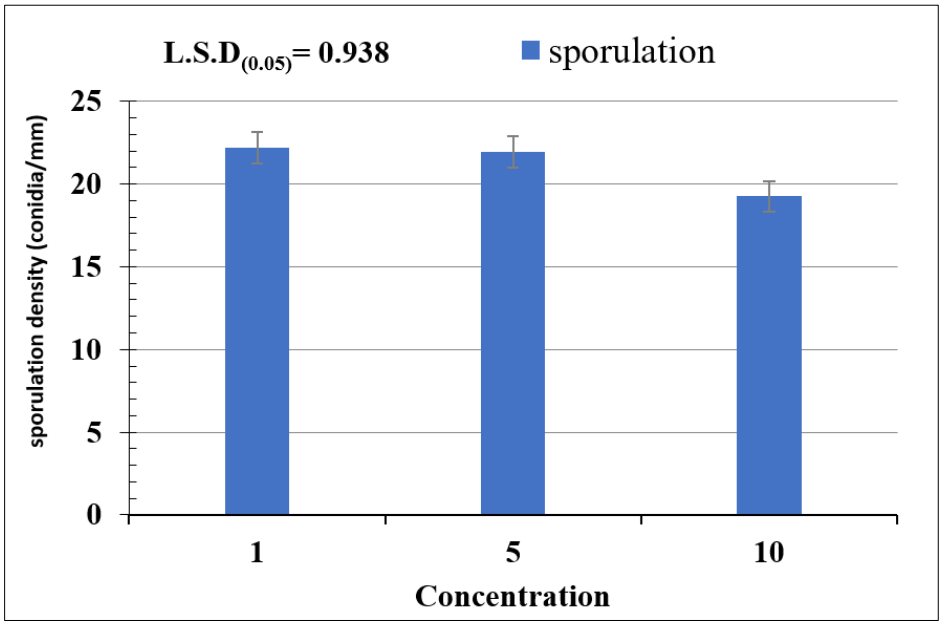

Fig. 2: Effect of concentration on the sporulation of *C.clavata*

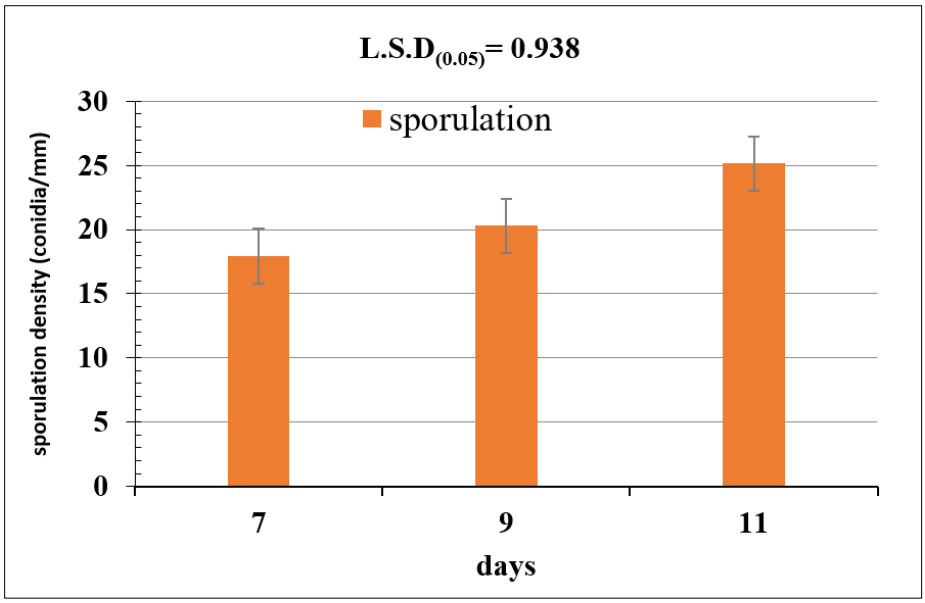

Fig. 3: Effect of time on the sporulation of *C.clavata*

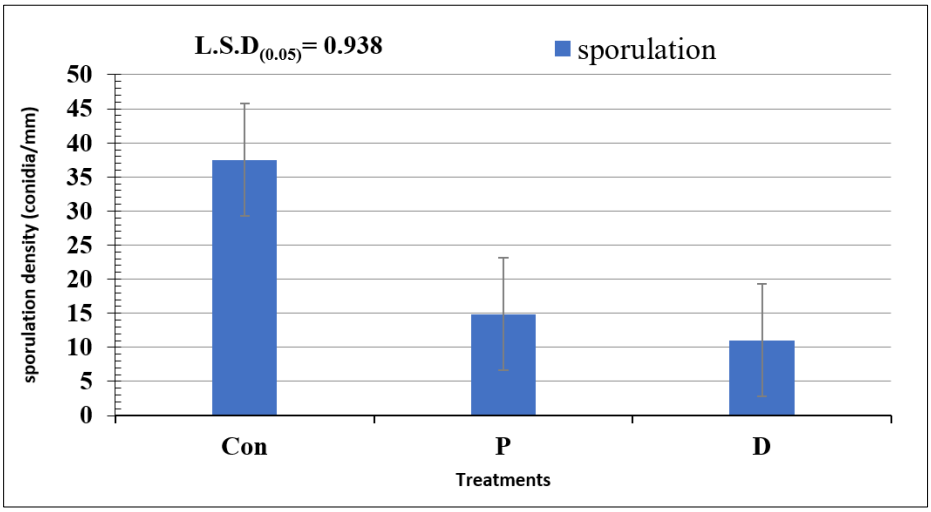

Fig. 4: Effect of treatments on the sporulation of *C.clavata*

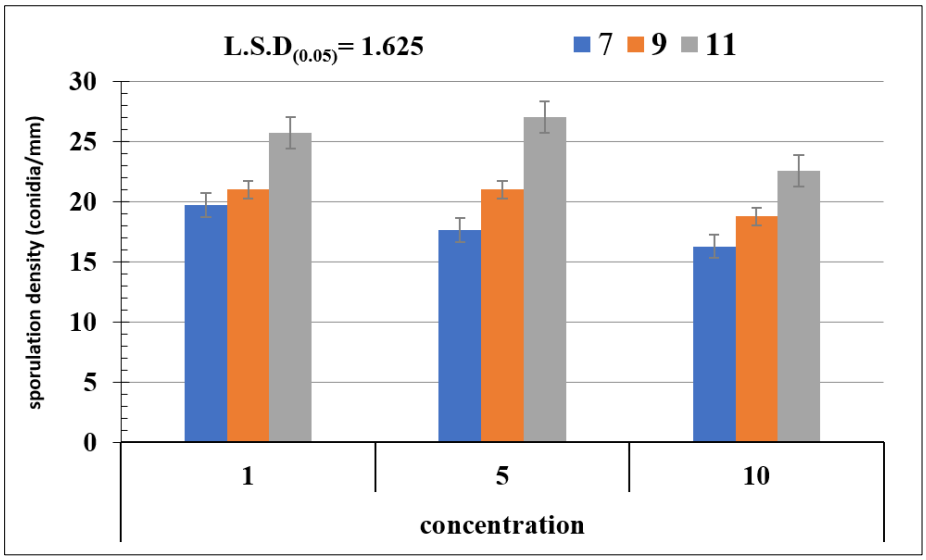

Fig. 5: Effect of concentration interaction with time on the sporulation of *C.clavata*

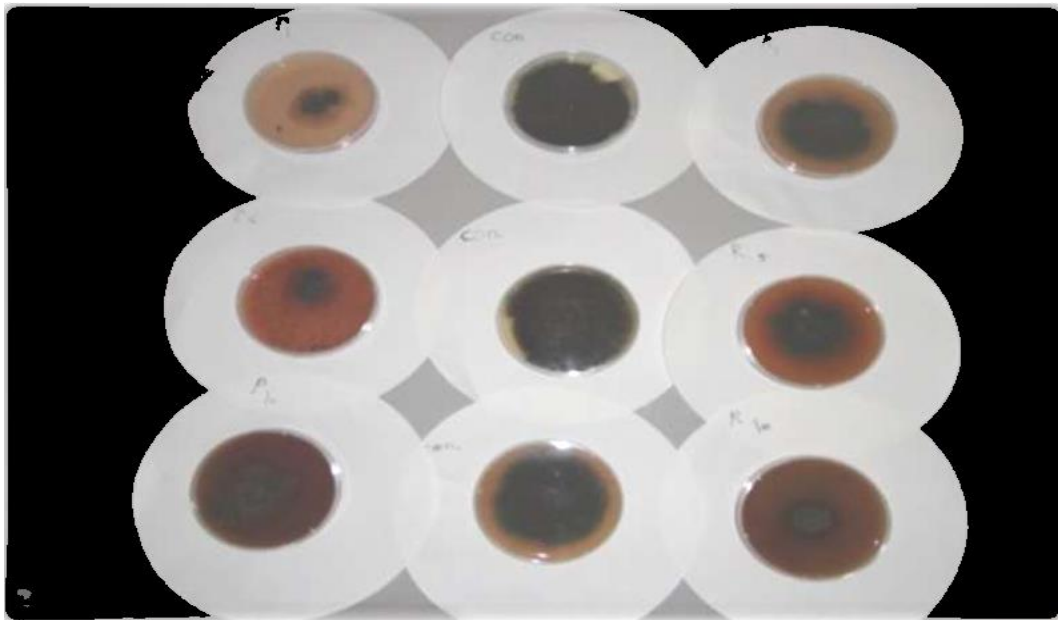

Fig. 6: Effect of treatment with yellow matter and pomegranate peels as well as control treatment on the radial growth of the fungus *C.clavata*

Table 1: Effect of interaction between time, treatments, and concentration on the diameter growth (cm) of the fungus *C.clavata*

Time (days)	concentration	treatments		
		Con	P	D
7	1	31.11	19.56	8.65
	5	31.11	12.22	9.78
	10	31.11	7.89	10.00
9	1	36.00	18.67	8.44
	5	36.00	13.89	13.22
	10	36.00	8.44	12.00
11	1	45.33	23.33	8.67
	5	45.33	19.00	16.78
	10	45.33	10.67	11.89
L.S.D(0.05)		2.815		

Antagonism between *curvularia clavata* & *Trichoderma orientale*, *curvularia clavata* & *Marasiemllus palmivorus/* Measure of the inhibition %, after 3 ,5 7 days of culture in Petri dish.

Treatment	3 days	5 days	7 days	Mean of Treatments
M.palmvorus vs *C. clavata*	37.5	50	87.5	58.33
T.orientale vs *C. clavata*	50	75	100	75.00
C. clavata control	0	0	0	0.00
Mean of time	29.17	41.67	62.50	
L.S.D. 0.05 Treatments = 10.268, Time = 10.268, Interaction = 15.329				

4-DISCUSSION

Calculation of sporulation of the fungus *Curvularia clavata*:

The results (Figure 1) showed that the concentration of (1 g/L) of the material collected from the growing tip of the palm tree had the highest effect on fungal germination, as it was (8.56) conidia/mm2 in all measurement stages compared to the control, which was (37.48) conidia/mm2, while the concentration of (10 g/L) had a clear effect after the first concentration, as it gave (11.8) conidia/mm2, unlike the pomegranate peels that were used to differentiate between it and the material collected.

From the growing tip of palm trees, where the treatment of pomegranate peels gave the highest effect on the germination of the fungus at a concentration of (10 g/L) where it gave (9) conidia/mm2 followed by a concentration of (5 g/L) where it gave (15.04) conidia/mm2 compared to the control which was at both concentrations (37.48) conidia/mm2

as shown in Figure (1) and the interaction had a significant effect on the germination of the fungus *C. clavata*. This may be due to the inhibitory enzyme capacity of the yellow substance collected from the growing tip of the palm tree, and pomegranate peels contain many antibiotics, including bacterial and fungal, and disinfectants against many pathogens that work to inhibit the growth of pathogens on humans such as Punica spp. (Aviram *et al.,* 2004 and Related *et al.,* 2007 and Gehad and Nahla, 2012).

Both (Kannan *et al.,* 2010, Eliana *et al.,* 2010, Saad Sabbar *et al.,* 2010, Viuda-Martos *et al.,* 2010, and Ephraim *et al.,* 2007) mentioned that the pomegranate pericarp contains many antibiotics and antioxidants such as uteolin, kaempferol, EA glycosides, EA, Pedunculagin, punicalin, phenolic punicalagins, gallic acid and other fatty acids, catechin, EGCG, quercetin, rutin and other flavonols, flavones, flavonones, anthocyanidins, acids, etc. It is noted from the results that there are significant differences between the experimental treatments, as the yellow material from the tops of palm trees was more effective than the pomegranate peel extract. This may be due to the inhibitory capacity of the yellow material from the tops of palm trees compared to the control treatment (Peccia and Hernandez, 2006). It is noted from Figure (2) that the concentrations have a significant effect on the germination of the fungus growing in Petri dishes, especially at a concentration of 10 g/L. This may be due to the increase in inhibitory materials with the increase in the amount of materials used. (Refai *et al.*, 2003) showed that increasing the concentrations of some materials and plant extracts increases their efficiency against some pathogenic agricultural pests. Figure (3) also shows that time has a significant effect on the effect on fungal germination in the different treatments, as the strongest effect of time was on the seventh day, followed by the ninth day. This is attributed to the inhibitory ability of the materials used on the growth and development of the fungus in time (Michel *et al.*, 2013).

Figure (4) shows that there are significant differences between the treatments, especially the yellow substance treatment, which had a greater effect on fungal germination. This may be due to the inhibitory ability of the yellow substance on fungal germination. Figure (5) also shows that the interaction of time with concentrations had a significant effect on the diameter growth of the fungus *C. clavata*, as significant differences were observed between the two factors on diameter growth. Figure (5) also shows that the interaction between treatments and time had a high effect on fungal germination, especially the yellow substance treatment. This may be due to the activity and effectiveness of the substance and its inhibitory ability on the growth and development of the fungus *C. clavata* (Alejandro *et al.,* 2009).

the results of the statistical analysis showed significant differences between the ability of the tested *T. orientale* and *M. palmivorus* isolates to oppose *C.clavata*. The highest growth inhibition rate for all pathogenic fungal isolates was recorded (1.5, 2) It reached 75% for *T. orientale* and 58% for *M. palmivorus*. Table 12. The appearance of inhibition zones was recorded at concentrations (1.5), and (2) and it is likely that Trichoderma spp. isolates can produce antagonistic substances that led to inhibition of the growth of pathogenic fungal isolates as well as competition for nutrients. (Amin *et al.,* 2010 & Subah, *et al.,* 2013)

5-CONCLUSION

Corn leaf spot C.*clavata* is a dangerous disease that causes severe damage to the corn crop. The disease has also been recorded in some weeds. Plant extracts were used to observe their effect on the fungus in the laboratory. Some fungi known in the field of biological control were also used, and they gave clear results in affecting the fungus and reducing its growth.

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