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Original Research Article

Two Isolates of *Alternaria alternata* can be used as a Biocontrol Agent Against Powdery Mildew of Squash Caused by *Podosphaera xanthii* in Greenhouses

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Abstract: Several experiments were conducted to evaluate the effectiveness of *Alternaria alternata* fungus isolates on powdery mildew disease on squash and to diagnose them according to the taxonomic keys of the *Alternaria spp* fungus genera. They were propagated to conduct experiments on them. The experiments showed a significant effect of the two isolates on the growth and germination of powdery mildew disease in squash. The two isolates also had a significant effect on reducing the formation of conidiophores. Powdery mildew disease was sprayed with a suspension of the two *A. alternata* fungus isolates, as it gave the greatest effect on the germination of powdery mildew disease, in addition to the number of conidiophores, followed by the spray treatment with reproductive units when compared to the control. The plants were also sprayed with the filtrate of the *A. alternata* fungus isolate, as it gave a tangible effect on the germination of powdery mildew disease, in addition to the number of conidiophores when measured by the control. Time also significantly affected the germination and number of conidiophores for powdery mildew disease.

Keywords: Alternaria alternata, Powdery mildew, Squash, Biocontrol, Fungal pathogens.

INTRODUCTION

The squash plant belongs to the Cucubitaceae family, which contains many economic and medium-sized plants spread in temperate regions of the world. The most important plants belonging to this family are cucumber, squash, watermelon, and others. These plants are affected by many dangerous diseases, including powdery mildew, which affects most species of this family, especially squash and cucumber crops, as it affects the quality of the fruits and their nutritional qualities. Severe infections also cause the plants to stop producing, as well as stop their growth and then die in severe infections, in addition to making the infected plants more susceptible to other diseases. Most squash varieties are sensitive to this disease, but the disease is less common in watermelon and watermelon due to the resistance enjoyed by watermelon and watermelon plants. The fungus *Podosphaera xanthii* and the fungus *Erisyphe cichoracearum* are the main causes of powdery mildew on squash, and the fungus *P. xanthii* is more widespread and dangerous than the second cause (McGrath, M.T., 2005; Davis *et al.*, 2008; Alejandro *et al.*, 2009, Armitage *et al.*, 2019). Powdery mildew symptoms are characterized by a white, flour-like growth on the upper surface of the leaf in the form of small spots that grow and expand and merge to cover the entire surface of the leaves from the upper and lower sides of the leaves, which leads to stopping the vital processes of the leaf and its shrinkage as a result of the withdrawal of water and nutrients from it as a result of the fungus feeding on it. The infected leaves become easily broken due to their lack of water and nutrients, which negatively affects the growth and development of plants, in addition to the deterioration and poor production (Zitter *et al.*, 1996; Braum *et al.*, 2002; Fernandez-Ortuno *et al.*, 2006). Powdery mildew is an obligate fungus and obtains its food from the living plant host by sending suckers to absorb water and nutrients from the host cells (Green And others, 2002, Sánchez *et al.*, 2020) and the disease spreads widely in greenhouses when the appropriate conditions are available for its growth and reproduction.

The fungus *P. xanthii* is characterized by the rapid development of its defenses against the fungicides used against it. For this reason and other reasons, including those related to the environment, health, and the danger of pesticides, many researchers have searched for other practices to combat diseases, including powdery mildew diseases, through safer methods in the environment, such as biological resistance, the use of extracts, or through integrated pest management, where the biological resistance fungus *Trichoderma harizanum* was used to combat powdery mildew on okra. It gave an effect rate of about 60% (Mecjrath, 1996; Mcjrath and Shiskoff, 1999; Gyung *et al.,* 2004; Bettiol *et al.,* 2008; Temur, 2009). The research aimed to prove the ability of the two isolates and their effect on the growth and reproduction of powdery mildew on squash.

MATERIALS AND METHODS

1 – Culture Media Used

1-1- Potato medium dextrose agar (PDA)

The medium was prepared by adding 39 g of the ready-made medium to a liter of sterile water according to the instructions of the Indian manufacturer (Himedia) and it was mixed well until homogeneous, then distributed into 250 ml flasks and their mouths were closed with a tight cotton stopper and sterilized in an autoclave at a temperature of 121 °C and a pressure of 15 pounds/inch2 for 20 minutes. After the sterilization period was over, the medium was left to cool and then stored in the refrigerator at 4 °C until use. Then this medium was prepared to isolate and purify the fungus *Alternaria alternata*.

1-2 - Potato Dextrose Broth (P.D.B.)

The medium was prepared by boiling 200 g of potato pieces after washing, peeling, and cutting them into small pieces in 500 ml of distilled water for 20-30 minutes. After the boiling period, the mixture was filtered using a piece of muslin cloth to obtain the extract. 20 g of dextrose sugar was dissolved in 500 ml of water, then the potato filtrate was added to it and the volume was completed to 1 liter and distributed in glass bottles, each with a capacity of 150 ml, tightly closed with cotton and sterilized as in the previous method. This medium was used to grow the *A. alternata* fungus isolate, then extract the filtrate formed after growing the fungus on the liquid culture medium and distributed in 13 glass bottles by placing 3 discs using a cork piercer with a disc diameter of 1 cm for each of the two isolates of *A. alternata* fungus and the one grown on the P.D.A. culture medium. At 5 days of age, incubated at 25 ± 2°C for 28 days and then refrigerated at 4°C (Dewan, 1989).

2 - Pathogenicity test on some economic plants:

Several plants were planted (pumpkin, cucumbers of both types, trout and squash, watermelon, watercress, beans, okra, broad beans, radish, garden cress, turnips, and celery, in addition to wheat and barley) in plastic anvils measuring 16 x 15 cm, each separately and in three replicates. After several weeks of planting, the plants were sprayed with reproductive units of each of the two isolates of the fungus *Alternaria alternata*. After that, a visual examination was conducted to observe whether infection had occurred. No infection was recorded on the mentioned plants.

3 - Propagation and preservation of powdery mildew inoculum on squash:

Cucurbita pepo L. plants were grown for the propagation of powdery mildew pathogen inoculum according to the method of Abood and Losel (2003). This is due to the large leaf area, rapid growth, and sensitivity to powdery mildew. Cucurbita plants at the age of the fourth true leaf grown in plastic houses were infected after taking infected cucurbit leaves from the field.

4 - Infection procedure for squash plants:

Squash seeds were planted in plastic pots measuring 16 x 15 cm with 2 kg of soil per pot in three replicates. The seeds were planted in the pots at a rate of 2 seeds per pot. Seven days after the emergence of seedlings, the plants were thinned to 1 plant/pot. After reaching the second true leaf, the plants were inoculated in 48 pots, and 3 pots were left for comparison with the fungal inoculum that was propagated as shown above (inoculum propagation paragraph). The most homogeneous plants in infection were selected as a source of inoculum that was about 7-9 days old. Inoculation was carried out artificially according to the method of both Eyal *et al*., (1968) and Abood and Losel, (2003) using a tower made of cardboard with a height of one meter and a width of one meter as well. Every 15 pots were inoculated with six leaves of the squash plants on which the fungus was propagated, which were almost homogeneous in size. They were then left for 5 minutes before lifting the barrier around the pot to allow the volatile spores to fall on the squash plants. The plants were then covered with nylon bags for 24 hours to provide the necessary humidity for spore germination.

5 - Calculate sporulation on squash plants:

Discs were taken from the leaves of the squash infected with powdery mildew on the day (7, 9, 11, 13) after infection, where five discs were taken from the first true leaf with a diameter of 1 cm using a cork piercer. 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. They were stirred gently for a few seconds to separate the mature conidia from the conidiophores that were to be counted. The discs were extracted from the solution, and then the numbers of spores formed were calculated using a Haemocytometer. The healthy spores were counted, and the spores stuck together and the deformed spores were excluded. Three readings were taken for each test tube (Bashi and Aust, 1986). One drop of the fixed solution containing the spores was placed and covered with a slide cover and calculated at a power of 10× with three replicates and three readings for each replicate. It was estimated that

Spore counts on days 7, 9, 11, and 13. The plants on which spores were to be calculated were exposed to an air stream 24 hours before taking the samples (Ghali and Al-Janabi, 1995).

6 - Calculating the number of conidia on squash plants:

The discs were taken according to the same method on the 9th day from the date of infection and were placed in a solution of alcohol and acetic acid in a ratio of (3:1). The discs were extracted after one day and placed in a solution of (Lactophenol bakrk acid or glycerol) after washing the discs with distilled water. The samples were incubated at a temperature of 60°C for one day and embedded in slides using Trypan blue 5% dye for half an hour. The numbers of conidia were measured using an eyepiece graticule and according to the method mentioned by Russell *et al.*, (1975), Carver and Carr, (1977), and Abood and Losel, (1991) to count the mature conidia in a single microscopic field that intersects with the axis and in two perpendicular directions.

7 - Diagnosis of the pathogen agent *A. alternata:*

The diagnosis was based on the diagnostic keys mentioned by (John and Roland, 2007).

8 - Spraying squash plants with fungus suspended: *A. alternata*:

Plants were sprayed with *A. alternata* reproductive units at a rate of 5 g of fungal inoculum/liter of sterile water to which 1 ml of sunflower oil and a drop of liquid soap were added for both isolates. After scraping the fungal inoculum grown in 7-day-old Petri dishes with a small brush Dewan, (1989). In addition to the control treatment, plants were sprayed with the same fungal suspension as well as with the fungal filtrate purified by milli-molecular or filter at a concentration of 100%.

9 – Effect of several concentrations of Thiophanate Methyl70% on the fungus for both isolates *A.alternata*

The results showed the ability of the fungus *A. alternata* both isolate (A&B), to grow in media containing the chemical fungicide (Thiophanate Methyl70%), which was used in three concentrations according to the recommendations of the producing company (1,1.5 and 2) ml per 1/liter of water, The results showed the growth of the cause of wilting of melons and watermelons in the first concentration recommended by the manufacturing company (Agrifochem / Origin: Australia).

RESULTS

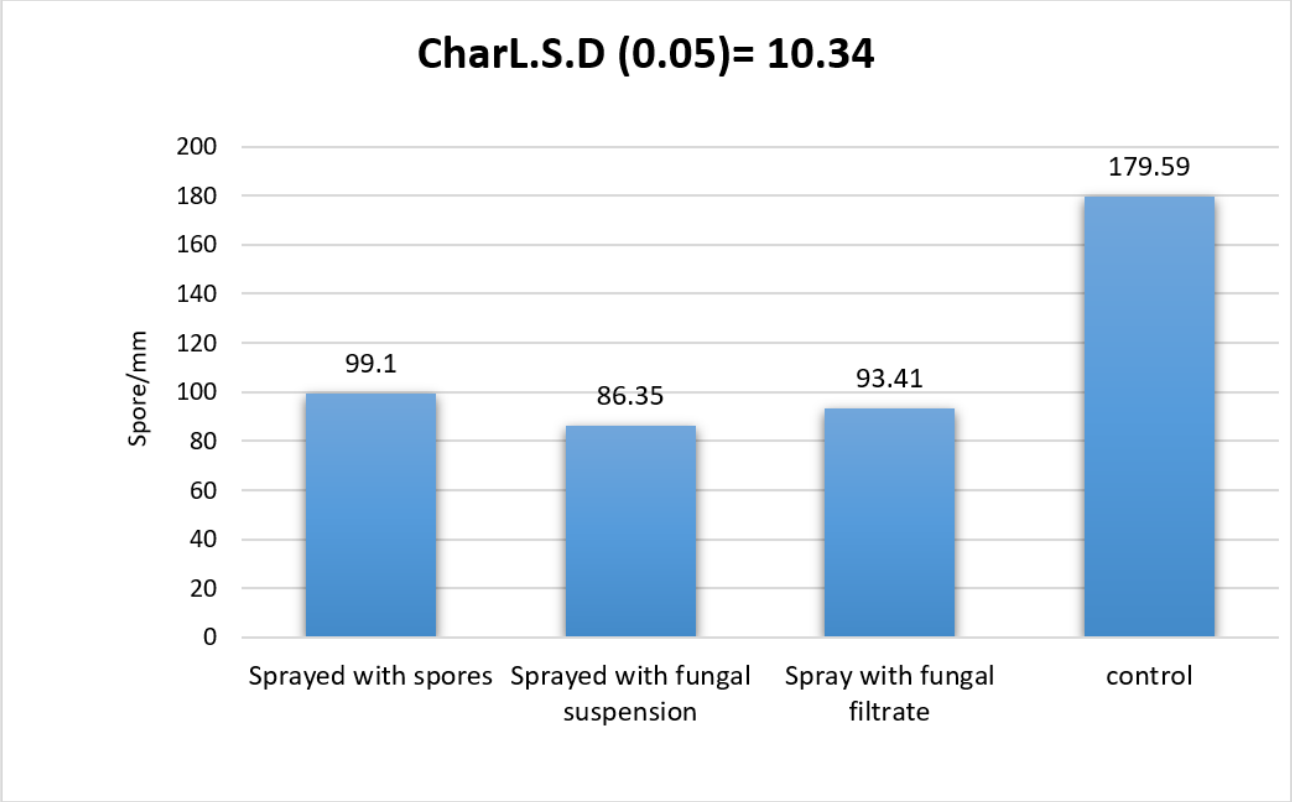

Figure 1: Shows the effect of different treatments on the sporulation effect of powdery mildew *P. xanthii* using the isolate *A. alternata*

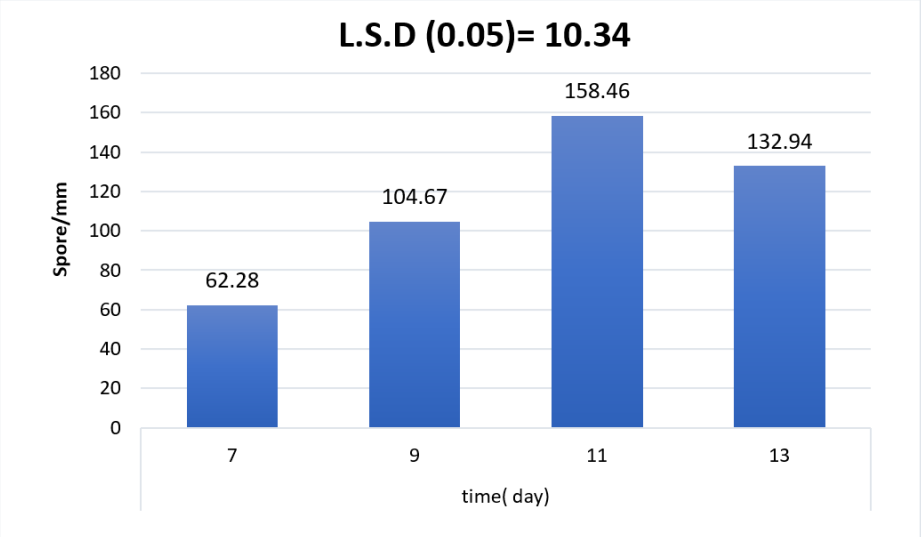

Figure 2: Shows the effect of time on the infection of powdery mildew *P. xanthii* using the isolate *A. alternata*

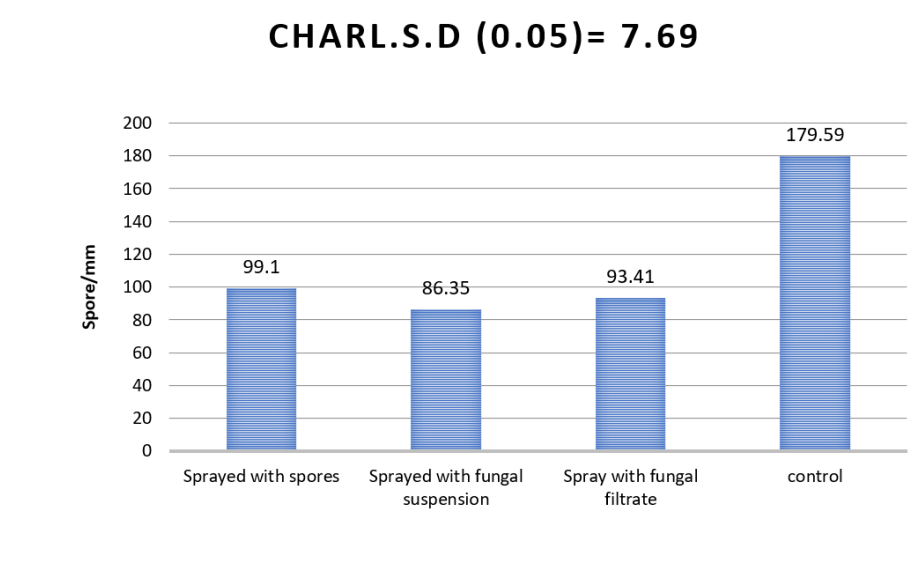

Figure 3: Shows the effect of different treatments on the sporulation effect of powdery mildew *P. xanthii* using the isolate B, *A. alternata*

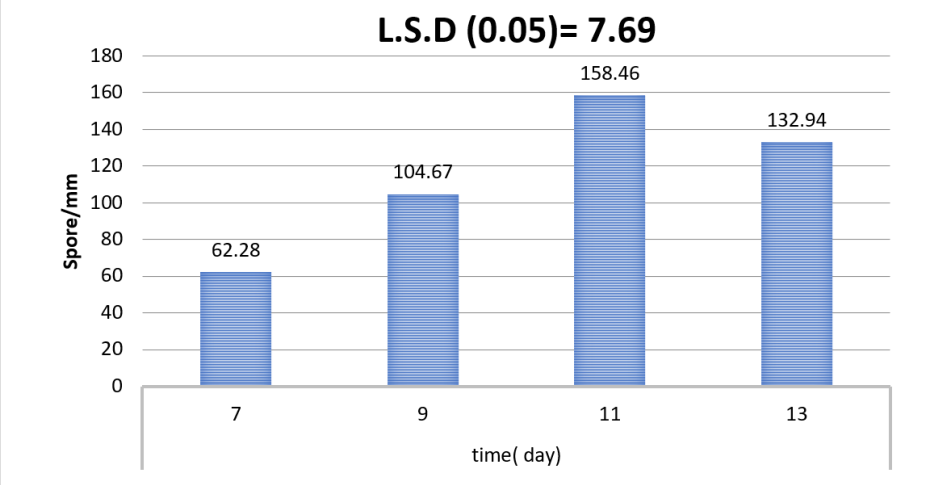

Figure 4: Shows the effect of time on the infection of powdery mildew *P. xanthii* using the isolate B, *A. alternata*

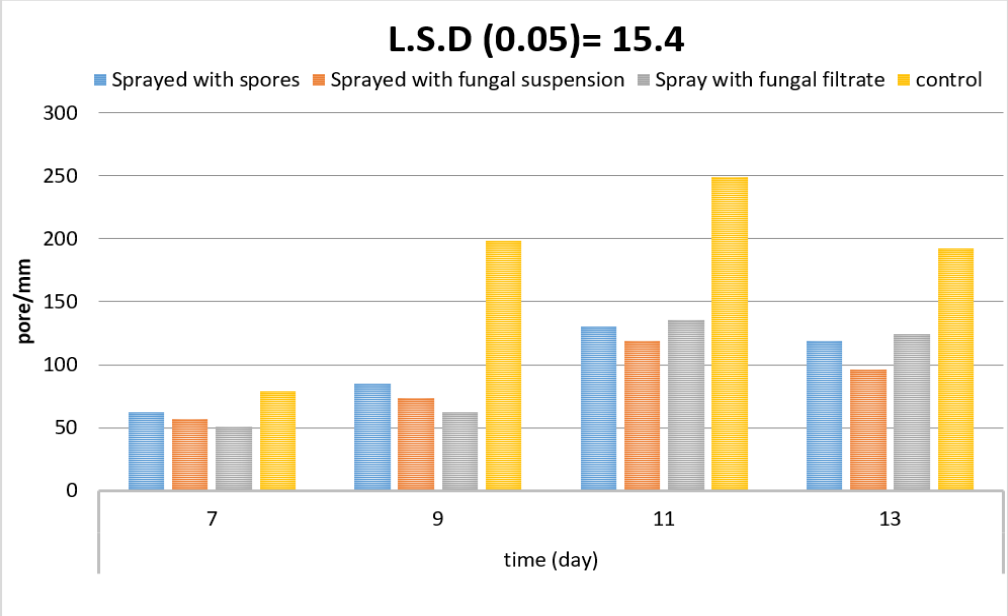

Figure 5: Shows the effect of interaction between treatments and time on the sporulation of the isolate (A). *A. alternata*

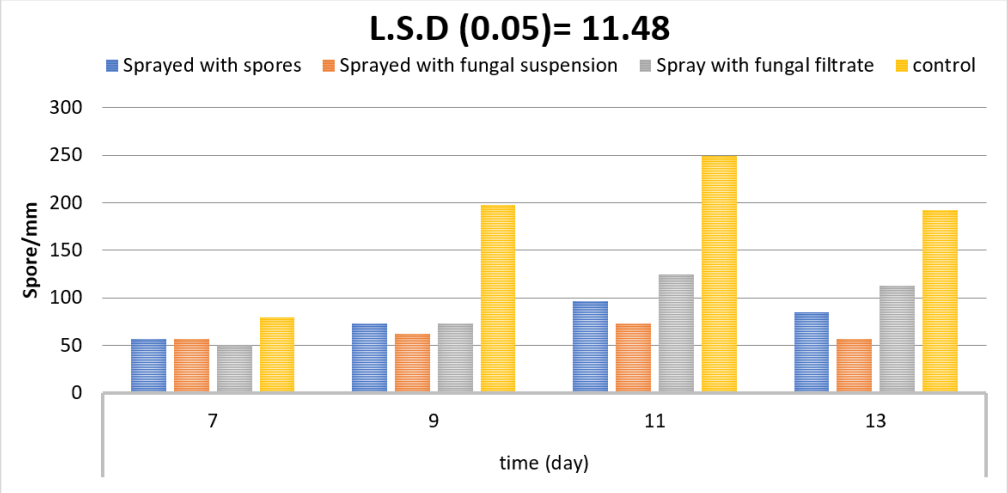

Figure 6: Shows the effect of interaction between treatments and time on the sporulation of the isolate (B) *A.alternata*

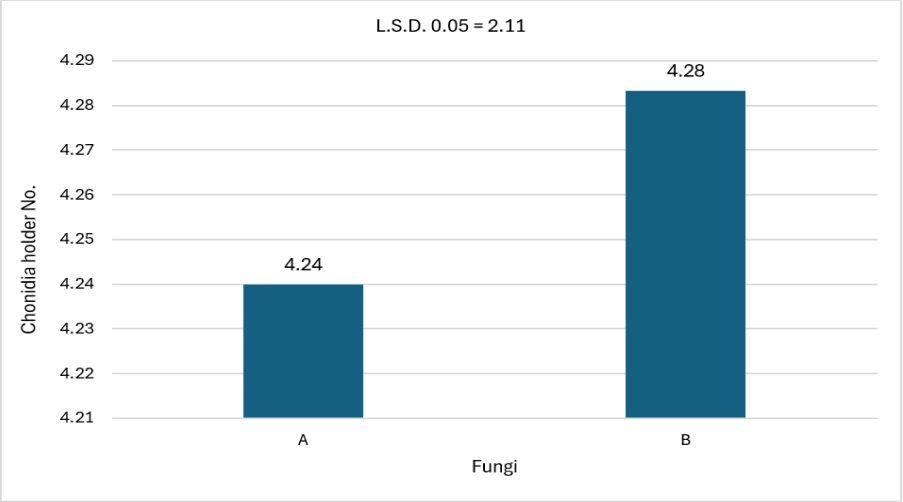

Figure 7: Shows the effect of the twoisolate (A &B) A.alternata on number conidia powdery mildew disease

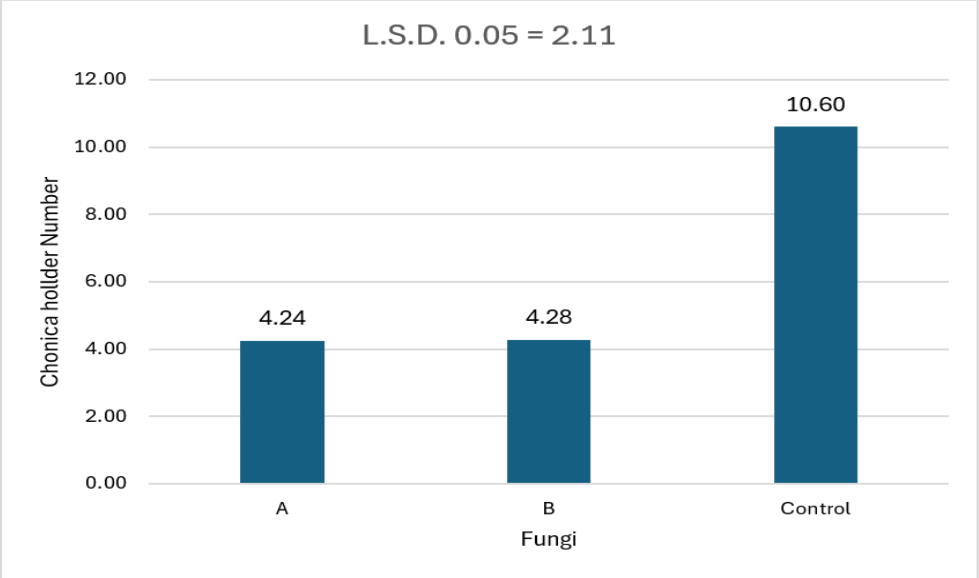

Figure 8: Shows the effect of the type of treatment on the number of conidia

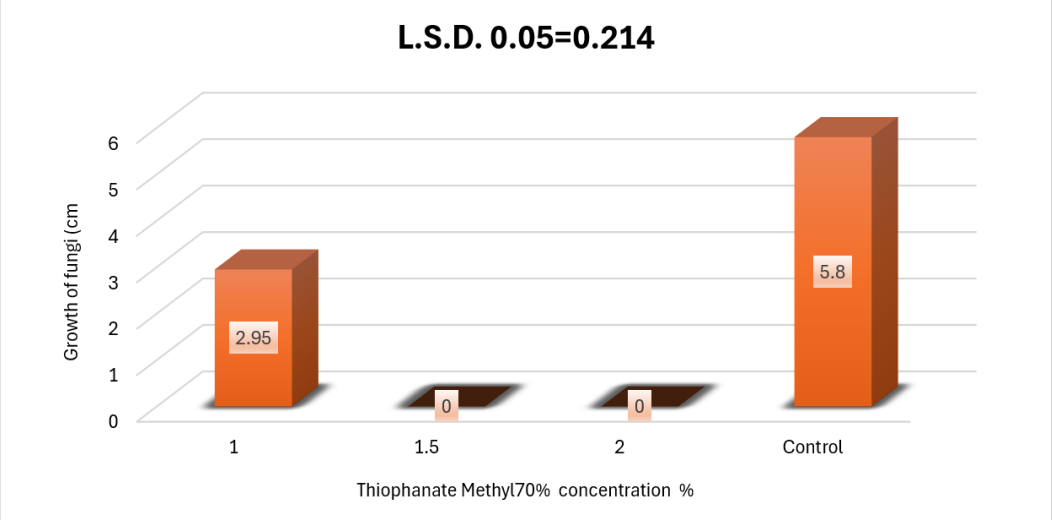

Figure 9: Shows the effect of different concentrations of the pesticide Thiophanate Methyl70% on the growth of powdery mildew fungus

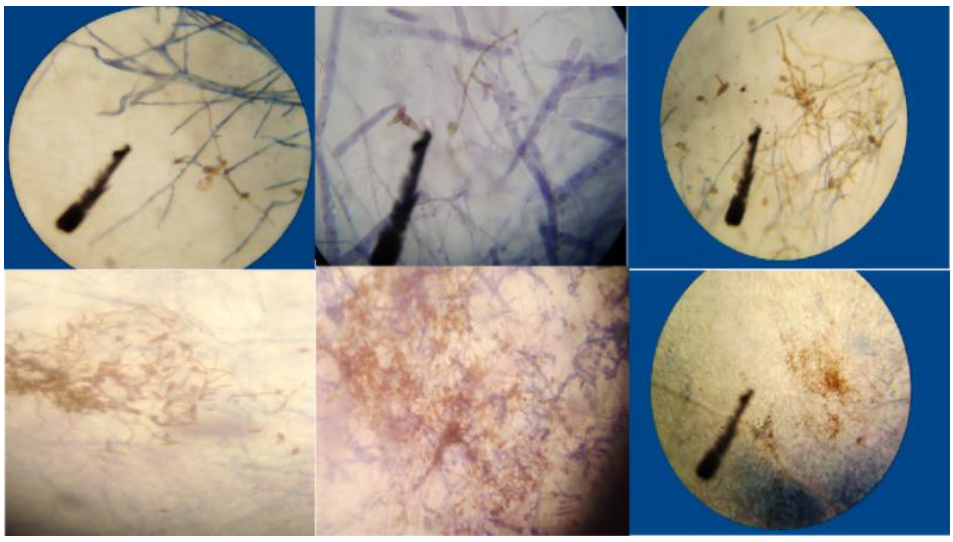

Figure 10: Parasitism of the fungus *A. alternata* on powdery mildew fungus on plant leaves

DISCUSSION

The results (Figures 1 and 3) showed that there was a significant effect of the treatments on the germination of powdery mildew fungus on squash *Podophaera xanthii* when measured by the control treatment, where the lowest germination rate was in the treatment of spraying with spore suspension for isolate (A), where it was 86.35 spores/mm2, followed by the treatment of spraying with filtrate only, where it reached 93.41 spores/mm2 compared to the control, which reached 179.59 spores/mm2. As for isolate (B), it had the greatest effect on the germination of powdery mildew fungus, where the treatment of spraying with spore suspension was 62.24 spores/mm2, followed by the treatment of spraying with reproductive units, where it was 90.56/mm2 compared to the control treatment, which reached 179.59 spores/mm2. It is noted that all the treatments of the two isolates had a significant effect on the germination and development of powdery mildew on squash, and it is believed that the reason for this is due to the parasitism of the two isolates of the fungus *A. alternata*. On powdery mildew fungus as noted in Figure (2, 4, 5, 6, 7, 8) in addition to the coverage of the two isolates of the fungus *A. alternata* on powdery mildew as a result of the growth of the two isolates on powdery mildew as shown in Figure (9 and 10) and it was also noted that the effect of parasitism increases as the growth of the powdery mildew fungus progresses. The reason may be because powdery mildew disease contains energy sources such as mannitol and trehalose which the fungus *A. alternata* needs, especially during the period of formation of conidiophores and conidia of the fungus Velez *et al.,* (2007, 2008) and Jennings *et al.,* (1998) as they mentioned that the fungus *A. alternata* does not need much mannitol during the germination period and formation of the appressorium. Still, it needs mannitol in advanced stages of its growth, which is the stage of formation of conidiophores and conidia, and this was noted on tobacco plants infected with the fungus *A. alternata*. Solomon *et al.,* (2006) and DeMers (2021). added that mannitol is required for the germination of scab disease or what is known as ray strike on wheat and also for brown spot *Alternaria sp* and *Botrytis cenaria*, where he confirmed their need for mannitol for germination. It is believed that the effect of spraying with the fungal filtrate of *A. alternata* is due to the presence of enzymes and chemical compounds contained in the filtrate, and this is observed through the results that the isolate (B) was more effective in the growth and germination of powdery mildew than the isolate (A). Perhaps the reason is due to the ferocity of the isolate (B) and its high parasitic ability. Some economic plants were also sprayed, which are (squash, cucumbers of both types, trout and cucumber, watermelon, watercress, beans, okra, broad beans, radish, garden cress, turnips, celery, in addition to wheat and barley plants), and no infection was recorded on the mentioned plants. Time also showed a significant effect for both isolates on the spore formation of powdery mildew when measured by the control, where it reached (10.347, and 7.691) in isolates A and B, respectively. It is also clear from the interaction that there are significant differences in the spore formation of powdery mildew, as is the case for conidia, where the greatest effect was in the spraying treatment with reproductive units, which reached (3.73) compared to the control, which reached (10.6). Also, significant effects were found through time, in addition to the interaction, and the reason is due to the intensification of the growth of the fungus *A. alternata* over the powdery mildew disease, in addition to the competition and secretion of enzymes Ribot *et al.,* (2007) and Ramires *et al.,* (2018). meaning that the cause grew in a concentration of 1,1.5 and 2) ml, while no growth was recorded in a concentration of (1.5 & 2) ml / 1 liter of water. The results were taken seven days after farming. The results in Figure (9) showed that the fungus caused complete inhibition of fungal growth at the concentrations 1.5 and 2%. alterations in cell division rate and/or cell death. This mechanism of action would be expected to exhibit the greatest adverse effects on rapidly dividing cells such as occurs during fetal development. The absence of any teratogenic effects by thiophanate-methyl could be attributable to its low potential to induce aneuploidy and polyploidy in mice and rats *in vivo* (Barale *et al* 1993; Proudlock 1999, Iori Imazaki *et al.,* 2006 & Gobashy *et al.,* 2018).

CONCLUSION

Powdery mildew disease on cucurbits is an economically important disease. The fungus *Alternaria alternata* was isolated after observing the presence of obscure areas in the center of the powdery mildew disease. Experiments gave clear results in the effect on the sporulation of the powdery mildew fungus and the number of conidiophores. The pesticide Topsin M (Thiophanate Methyl 70%) was used at different concentrations to study the extent of its effect on the growth and development of the fungus.

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