First report of *Chaetomium madrasense* causing root rot Disease in *Calendula officinalis* and its biological and chemical control

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Abstract
The following study was conducted the Faculty of Agriculture/Karbala University to isolate and diagnose the cause of the disease of seedling death and root rot of chrysanthemum, test its pathogenicity and molecular diagnosis, and control it chemically using the pesticide Beltanol and biologically using the fungus *Trichoderma koningiopsis*. The Hungarian diagnostic results indicated that the pathogen is the fungus Chaetomium madrasense, and it was registered in the US Genebank serial number PP467627.1. The results showed that the pathogenic fungus *Chaetomium madrasense* inhibited the germination of chrysanthemum seeds in the culture medium and in plastic pots by 100%. The complementary treatment Chaetomium madrasense + Trichoderma koningiopsis + Beltanol was superior in reducing the percentage of infection and its severity by the pathogenic fungus, which reached 12.33 and 5.66%, respectively, compared to the treatment of the pathogenic fungus alone, which reached 100 and 55.66%, respectively.
Keywords: chrysanthemum, fungi, *Trichoderma koningiopsis*, Beltanol.

Introduction

Calendula officinalis L. is a beautiful ornamental plant and one of the most important plants used in the food and cosmetics industry [10]. Chrysanthemum flowers are characterized by their yellow color and radial shape, and are rich in carotenoids, ketones, flavonoids, amino acids, and volatile oils [14]. Chamomile syrup is used to reduce fever and illnesses. [29] [34]. The chrysanthemum flower extract contains 15 kinds of amino acids, including proline, and amino acid content [7]. Chrysanthemum flowers contain acids including lauric and palmitic acids, phospholipids, and neutral fats. The yellow and orange color of the flowers is due to the presence of carotenoids in the flower petals. Yellow flowers contain nineteen types of carotenoids, and the orange flowers contain ten unique types of carotenoids. These ten carotenoids have the maximum visible absorption of ultraviolet rays [12].
Chamomile contains many active antibacterial and antifungal properties and has been used to treat wounds, burns, ulcers, skin infections, and eczema [26]. The chrysanthemum plant is affected by many diseases that may lead to the appearance of different symptoms, including seed rot, weak germination, root rot, wilting and sometimes death of the plant, despite its importance, chrysanthemum plants are susceptible to several fungal diseases that affect seed germination and seedling growth, including the fungus Chaetomium and others capable of inhibiting plant growth.
Due to the lack of studies on the diseases that affect this plant in Iraq, a study on the identification of isolated fungi causing root rot disease and death of chrysanthemum seedlings and combat them chemically and biologically.

Materials and working methods

Sample collection and fungal isolation

Collection roots of the chrysanthemum (*Calendarula officinalis*) taken from plants, the disease, and rotting of its roots. These samples were collected from the Faculty of

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Agriculture, University of Karbala, during the 2023-2024 season. The roots were washed well with water and then cut into small pieces (0.5-1) cm in size and sterilize with sodium hypochlorite for two minutes, wash with water, dry and plant in PDA medium to which the antibiotic Amoxillin was added at a concentration of 125 mg/L. The dishes were placed in an incubator. After growing the isolated fungi, they were identified morphologically and microscopically using taxonomic key[15,31]

Testing the pathogenicity of isolated fungi on chrysanthemum seeds in WA (Water and Agar) culture medium

Two fungal isolates were used in the pathogenicity test. The pathogens were Taken from plant roots of the chrysanthemum plant using sterile agar and water agar with the antibiotic amoxicillin at a rate of 125 mg/L. They were inoculated with a 5 mm diameter disk from a colony containing pure fungi in the nutrient medium that was previously counted. The dishes were incubated in an incubator for three days at a temperature of 25 ± 2 °C. Then, chrysanthemum seeds that were sterilized from the outside were placed at a rate of 10 seeds. Three replicates were used for each fungus and then sent to the incubator for seven days, and the germination percentage was calculated using the equation. [23]

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100\%$$

Also, the percentage of non-germinating seeds was calculated using the About equation [1].

$$\text{Percentage of inhibition} = \frac{\text{seeds in comparison - seeds in treatment}}{\text{Number of seeds germinated in comparison}} \times \%100$$

Testing the pathogenicity of isolated fungi on the growth of chrysanthemum seeds in polyethylene pots in greenhouses

The experiment used soil consisting of equal amounts of sterilized soil and peat moss, which was divided into plastic planting boxes, and millet (*Panicum miliaceum*) was added to the moist soil [9]. At a quantity of 1%, after two days, 6 chrysanthemum seeds were added to each pot, and the experiment was analyzed within (CRD).

DNA analysis of fungi the most pathogenic

PCR technology was used to identify the type of fungus causing the death of chrysanthemum plants [9]. Take 2 microliters of fungal DNA using the kit DNeasy commercial methods and also primers ITS1 and ITS4 -5' (TCC GTA GGT GAA CCT GCG G) 3', and (TCC TCC GCT TAT TGA TAT GC) 3' 5′ (White *et al.*,1990). And also used the genes ACT-512 F and ACT-783 R. (5′-ATG TGC AAG GCC GGT TTC GC-3′, 5′-TAC GAG TCC TTC TGG CCC AT-3′).

Testing antagonistic ability of biological agent *Trichoderma koningiopsis* against the fungus *Chaetomium madrasense* isolated from the roots of the chrysanthemum plant on PDA culture medium.

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The antimicrobial activity of T. koningiopsis against the pathogenic fungus was tested using the dual culture method [3]. Petri dishes were divided into 2 parts, with a fungus in the middle of each one. After the growth of the fungi in the control, the inhibitory efficiency and antagonistic capacity of the biological agent were calculated based on the [5].

Next scale :

Degree 1: Bio-resistant fungi grow to fill the dish.

Degree 2: The biological agent covers most of the area plate.

Grade 3: The biological agent covers half the area.

Grade 4: the pathogenic fungus grows on most of the plate.

Grade 5: The pathogenic fungus covers the entire plate.

The biological factor is effective if the degree of antagonism is 1 or 2. The method of determining the prevention of fungal growth [25] used by the [1] to measure the percentage of inhibition.

$$\text{Percentage inhibition} = \frac{\text{Growth rate in comparison} - \text{Growth rate in treatment}}{\text{Growth rate in comparison}} \times 100$$

The antagonistic activity of the biological agent was determined based on the percentage of inhibition and using the [28] scale (Table 1).

Table (1) Antagonistic activity of biological agent

Percentage of inhibition	Degree of effectiveness
0 %	Not effective
20 %More than 0 to	Little effectiveness
%50 More than 20 to	Average effectiveness
And less 50 More than100 %	Effective
100 %	Very effective

Evaluation of the efficiency of the pesticides Beltanol, Metchazole against the pathogenic fungus *Chaetomium madrasense* causing root rot and death of chrysanthemum seedlings in PDA culture medium.

Evaluation of the efficiency of the pesticides Beltanol, Metchazole against the pathogenic fungus *Chaetomium madrasense* causing root rot and death of chrysanthemum seedlings in PDA culture medium

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Table 2: Concentrations of fungicides used to control the fungus *Chaetomium madrasense*, which causes root rot and death of chrysanthemum seedlings in the PDA culture medium

Name of pesticide	Recommended concentration/ 1L	Concentration used/ 1L	picture of pesticide
Beltanol	Ml0.75 ,1 ,1.25	Ml 1	Water soluble concentrate
Metchazole	Gm0.75 ,1 ,1.25	Gm 1	Spreadable granules

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Glass flasks were prepared, each containing 250 ml of sterile PDA medium. The pesticides Beltanol and Metchazole were added to the flasks at the concentrations shown in Table 2 for each pesticide separately. Then the flasks were shaken well and the media with the pesticides were poured into sterile Petri dishes. After the medium solidified, it was inoculated by taking a 0.5 cm diameter disk from the edge of each fungal colony growing on the PDA medium. The experiment according to the completely randomized design and with the same number of replicates.

Evaluation of the efficiency pesticide Beltanol and the bio-agent Trichoderma koningiopsis and their interaction against the fungus Chaetomium madrasense, that caused death of chrysanthemum seedlings in greenhouse conditions.

This experiment was conducted in the plastic house, using 1 kg plastic pots containing sterilized mixed soil using an autoclave. The pathogenic fungus was added to the soil and then the vaccine was mixed well with the soil to homogeneity. The pots were watered well and covered with perforated polyethylene bags for 48 hours, after which chrysanthemum seeds were planted at a rate of 6 seeds/pot. The treatments were applied at a rate of three replicates for each, as shown below:

1- Correct comparison
2- C.madrasense is a solitary pathogenic fungus.
3- Beltanol
4- T. koningiopsis
5- T. koningiopsis + C. madrasense
6- Beltanol + C.madrasense
7- Beltanol+T.koningiopsis+C.madrasense

After 30 days of implementing the experiment, the infection rate was calculated according to the following equation:

$$\text{Percentage of infection} = \frac{\text{Number of infected plants}}{\text{Total number of plants studied}} \times 100\%$$

The disease key was relied upon to assess the severity of root rot infection as stated in [6] and [17] as follows:

0 = healthy roots
1 = Secondary root discoloration (rot)
2 = Discoloration of secondary roots and part of primary roots
3 = Root discoloration without stem base rot
4 = plant death

Conduct a severity assessment as in (McKinney, 1923) equation mentioned:

$$\text{Infection severity \%} = \frac{\text{Total (the plants in grade x grade number)}}{\text{Total plants x highest grade}} \times 100$$

Results and discussion

Phenotypic diagnosis of fungi pathogens of chrysanthemum

Two isolates of the chrysanthemum plant were found, belonging to the genus Chaetomium sp. (1). and an isolate

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belonging to the genus Fusarium sp. (2). According to the observation of mushrooms at the beginning, as well as the microscopic characteristics of each ([15,16]

Testing the fungi isolated from chrysanthemum seeds in the laboratory on WA (Water Agar) culture medium

From the Test the chrysanthemum plant in the laboratory (Table 3 and Figure 1) seems isolate 1, was High 2 of the chrysanthemum seeds, which reached 0%, and didn't grow for 100%, which reached 100% and 0.00%. the isolated R2, the germination percentage and the inhibition percentage were 70% and 30%, respectively.

Table 3. Pathogenicity of fungi isolated from chrysanthemum seeds in the laboratory on WA culture medium.

Transaction	For germination%	Inhibition%
Comparison	100	0.0
Isolation 1	0.0	100
Isolation 2	70.0	30.0
LSD0.05	0.031	0.022

* Three repetitions of each.

Figure (1) Testing the pathogenicity of the isolated fungus on chrysanthemum seeds in the laboratory on WA culture medium. A. Comparison without fungus B- Isolation

Testing the pathogenicity of isolated fungi on germinating chrysanthemum seeds in plastic pots

In (Table 4, Figure 2) was superior in reducing the germination of chrysanthemum seeds, it was germination 0.00% and a growth 100%, which reached 100% and 0.00, respectively. While the results showed that isolate 2 Caused a lack of growth of 83.33% and 16.66%, found many postgraduate studies were found for her, A group of fungi Chaetomium sp. that have the ability to infect a number of plant families and cause disease symptoms to appear on them, as [21] found the fungus C.globosum causes leaf spot disease on pomegranate in Pakistan. [30] found that the same species causes leaf spot disease on eggplant in India [3] reported This is a cause of the same disease on Averrhoa carambola in India. The fungus Chetomium sp. belongs to the sac fungi and forms fruiting bodies of the Perithecia type. Most strains of this fungus are used in biological control of plant pathogens. In this study, the fungus showed a high pathogenicity in infecting chrysanthemum plants. This may be attributed to the

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difference in the strain and its ability to secrete enzymes that decompose pectin and cellulose, including pectinase,phosphatase,cellulase, methylesterase, pectinase, and pectinmethylhydrase, which have a significant effect on the pathogenicity of fungi, in addition to the ability of these fungi to produce some toxins of a phenolic and glycosidic nature. Based on the results of the laboratory experiment and the greenhouse experiment, isolate who takes for diagnosis and completion of other experiments.

Table 4. Testing of pathogenicity of fungal isolates in germinating chrysanthemum seeds in plastic pots under greenhouse conditions.

Transaction	For germination%	Inhibition%
Comparison	*100	0.0
Isolation 1	0.0	100
Isolation 2	83.33	16.67
LSD0.05	0.034	0.025

Figure 2. Testing the pathogenicity of isolated fungi on germinating chrysanthemum seeds in plastic
pots under greenhouse conditions.
A- Isolation R1 B- Isolation R2 C- Correct comparison

Molecular diagnosis of pathogenic fungal isolates

The results showed that the DNA of the fungus isolated in this study had a successful amplification of the r DNA - ITS region using primers (ITS1-ITS4), which produced a single double-stranded amplification of 500-1000 bp in length. The results of bioinformatic analysis of the ITS region sequence using the BLAST program and the

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preliminary diagnosis based on phenotypic characteristics confirmed that the most pathogenic fungus isolated in this study is *Chaetomium madrasense*, the sequences of the identified genus were deposited in GenBank, and a sequence code for the genus isolated in this study was obtained. The genetic tree was drawn and compared with other isolates registered in GenBank (Table 5 and Figures 3 and 4).

Table (5) The identified fungal isolates preserved in the Gen Bank database with their serial code.

N	Name fungi	Serial number
1	*Chaetomium madrasense*	PP467627.1

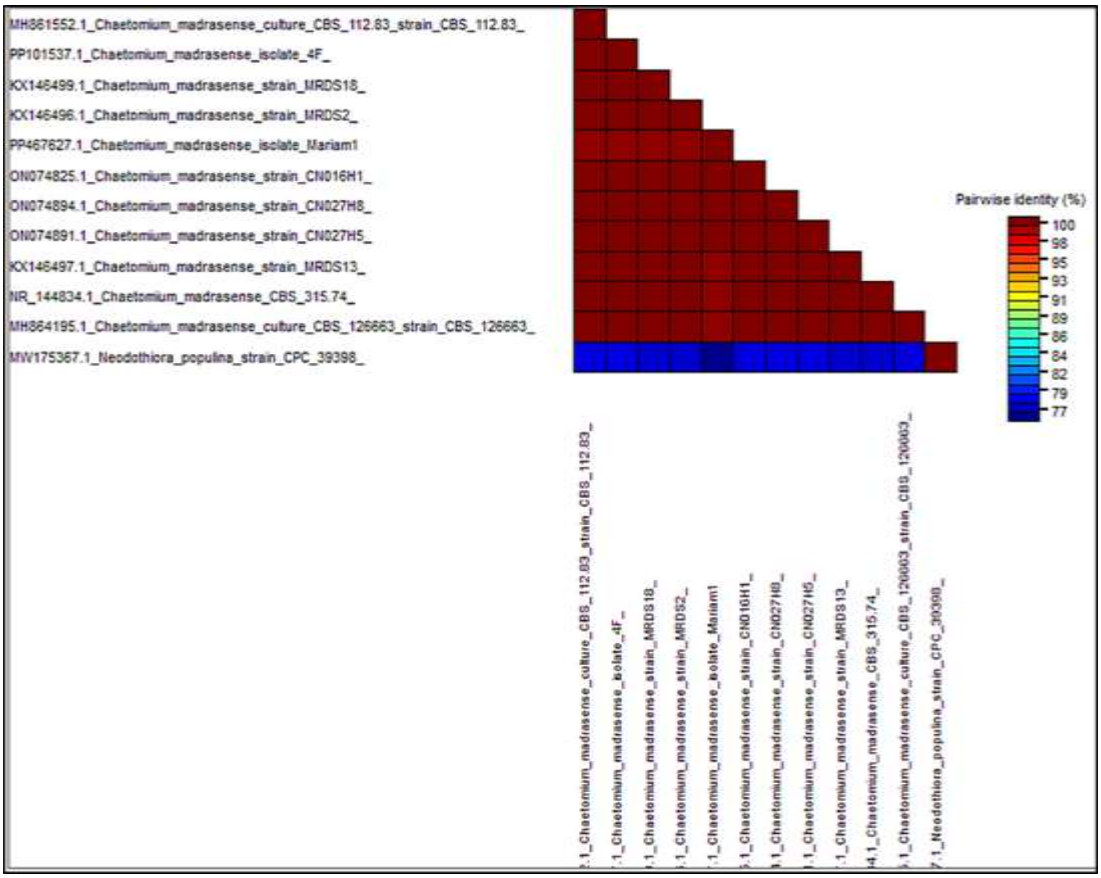

Figure (3): The percentage of similarity between the Chaetomium madrasense isolate Mariam-1 (marked with a black dot) and the corresponding global isolates of the same fungus based on sequences of the ITS-rDNA. This figure was created using the Sequence Demarcation Tool version 1.2 program.

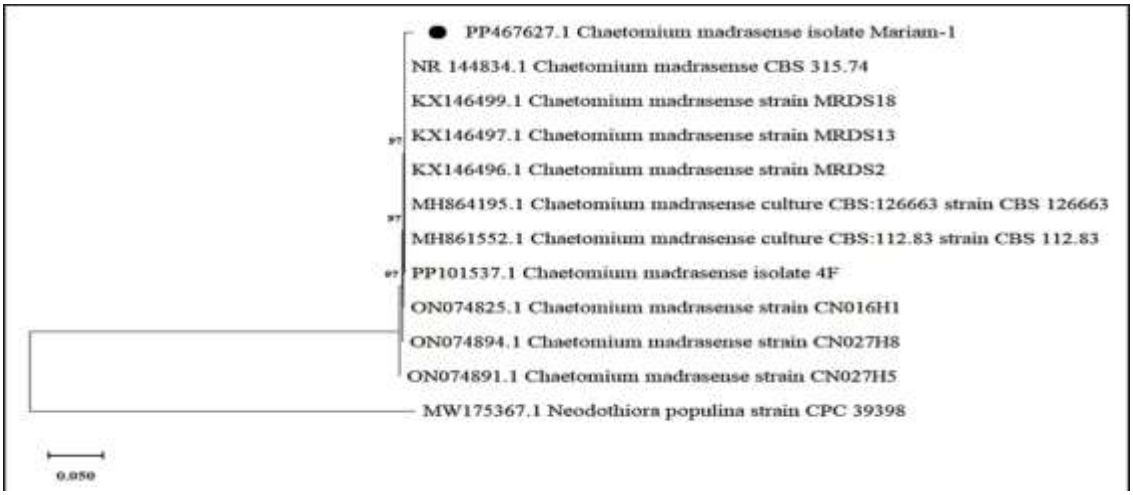

Figure (4): The genetic tree of the fungus *Chaetomium madrasense* isolate Mariam-1 (marked with a black dot), which was constructed based on global strains from the GenBank data container with using the neighbor-joining method to determine the genetic distances.

Testing the antagonistic ability of the biological agent Trichoderma koningiopsis against the fungus Chaetomium madrasense isolated from the roots of the chrysanthemum plant in the laboratory.

Figure 5 shows the effectiveness of the fungus T.koningiopsis in controlling the pathogenic fungus *Chaetomium madrasense*, with a rate of 100% compared to the treatment, which had an inhibition rate of 0.00%, thus achieving a score of 1 on the Bell scale [5]. This study was consistent with many studies that proved the effectiveness of the fungus Konigsiopsis T. in controlling many plant pathogenic fungi, including [11], where the effectiveness of the fungus was T. Koningiopsis was observed in controlling the two pathogenic fungi R. solani and F. oxysporum, as the bioresistance fungus was significantly superior in controlling the fungus F. oxysporum with an inhibition rate of 100%, followed by the fungus R. solani, which had an inhibition rate of 88.63%, which had an inhibition rate of 0.00%.

Figure (5): The antagonistic ability of the biological agent Trichoderma koningiopsis against the fungus Chaetomium madrasense isolated from the roots of the chrysanthemum plant in the laboratory on the PDA culture medium. A = Chaetomium madrasense fungus, B = pathogenic fungus + T. koningiopsis

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Evaluation of the efficiency of the pesticides Beltanol, Metchazole against the pathogenic fungus Chaetomium madrasense causing root rot and death of chrysanthemum seedlings in PDA culture medium.

Table 6 and Figure 5 showed that the chemical pesticides Beltanol and Metchazole, used to control the pathogenic fungus *Chaetomium madrasense* in the laboratory, inhibited the fungus's growth by poisoning the culture medium PDA. The two pesticides varied in their effect on the pathogenic fungus, as the chemical pesticide Beltanol was superior. All the concentrations used of this pesticide (0.75, 1.0, and 1.25 ml/liter) led to inhibition of the pathogenic fungus growth by 100% (Figure 5) compared to the comparison treatment, in which the inhibition rate was 0.0%. In contrast, the concentrations used of the chemical pesticide Metchazol, which are 0.75, 1.0, 1.25 g/liter, led to inhibition of the pathogenic fungus by 73.03, 78.50,87.33% respectively. These concentrations differed significantly in their inhibition of the pathogenic fungus.

The results of this study agree with [24], as it was found that complete inhibition of the fungus *Fusarium solani* occurred. When using the pesticide Beltanol (8-Hydroxyquinoline) at the same concentrations used in this study, while the inhibition rate reached 0% in the comparison treatment, while the inhibition rate with the pesticide Metchazol reached 74.03%, the fungi *Pyricularia oryzae*, *Bipolaris oryzae*, *Curvularia lunata* and *Fusarium incartanum* that cause rice diseases, this pesticide caused low inhibition of fungi, reaching 32-33% As specified dose (Hymenozol) was the least effective chemical pesticide used in the study in inhibiting of F. oxysporum, the inhibition rate did not exceed 75% when used at a 77.70%, 85.50%, respectively. This study also agrees with [17], whose study results showed the efficiency of the pesticide Beltanol and its achievement of a 100% inhibition rate for the growth of pathogenic fungi Rs.solani (R16), F. solani (F3),and *Ectophoma multirostrata* (E2) when used at concentrations lower and higher than the recommended concentration. These results agree with many studies that showed Beltanol pesticide prevents fungal growth of a large number fungal and bacterial plant pathogens [2].

The effect of the chemical pesticide Beltanol on pathogenic fungi may be attributed to its ability to form due to the presence of chelating materials, and then being released and killing the pathogen [20] [27]. The active ingredient in the pesticide, 8-Hydroxyquinoline, is highly efficient and eliminates a range of fungi.

One of the substances derived from this active ingredient has proven its inhibitory efficiency against the fungi *Sclerotinia sclerotiorum*, *Fusarium graminearum*, *Magnaporthe oryzae*, and Ilyonectria liriodendra. The effect of this substance on fungi is due to causing deformities in fungal cells, changing the permeability of the cell membrane, leaking its contents to the outside, and inhibiting the formation and germination of sclerotia [33] [8]. These results do not agree with what was found by Kongcharoen et al. [13] when using the active ingredient in Tabsen Thiophase methes against compared to other pesticides such as Mancozab, Fluopyrom and Carbendazin, which were more effective in inhibiting the growth of pathogenic fungi. [18] found that the use of Metchazole concentration of 1000 ppm. Based on the results of this experiment, Beltanol was selected for use in subsequent experiments

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Table (6) Evaluation of the efficiency of the pesticides Beltanol, Metchazole against the pathogenic fungus *Chaetomium madrasense* causing root rot and death of chrysanthemum seedlings in PDA.

Pesticide / *Concentrate	%For Fungi inhibition Pesticide Beltanol	%For Fungi inhibition Pesticide Metchazol	Concentrate rate
Control	0.00	0.0	0.00
0.75	100.00	73.03	86.51
1.0	100.00	78.50	89.25
1.25	100.00	87.33	93.66
Fungi Rate	75.00	60.05	
L.S.D0.05	The Concentrate	The Pesticide	
	0.8333	0.7217	

*mL/liter for the first pesticide, g/liter for the second pesticide.

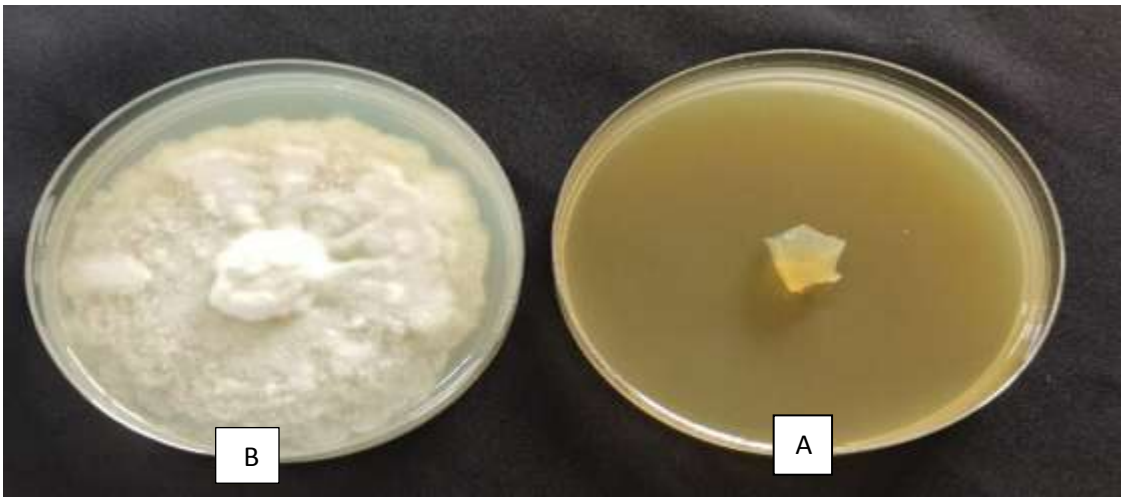

Figure 6. Evaluation of the efficiency of the pesticide Beltanol against the fungus Chaetomium madrasense causes death of chrysanthemum seedlings in PDA culture medium.
A=fungus +Pesticide B= pathogenic fungus alone

Efficiency of the pesticide Beltanol and the bioagent and their interaction Chaetomium madrasense, death of chrysanthemum seedlings

Table 7 used in this experiment led to a reduction in the percentage and severity of infection with root rot disease and death of chrysanthemum seedlings caused by the fungus C. madrasense, as the treatment madrasense C. + Beltanol + T. koningiopsis was superior, which reaching 12.33 and 5.66%, followed by the treatment C. madrasense + Beltanol, which reached 19.66 and 12.66, respectively. This treatment

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differed significantly from the treatment Madrasense C. + T. koningiopsis, reducing disease, reaching 100 and 55.66% in the pathogenic fungus alone, respectively. The The active ingredient in the pesticide, 8-Hydroxyquinoline, is effective against a wide range of plant-pathogenic fungi. One of the substances derived from this active ingredient has proven its inhibitory efficiency against the fungi *Sclerotinia sclerotiorum*, *Fusarium graminearum*, *Magnaporthe oryzae*, and Ilyonectria liriodendra. The effect of this substance on fungi is due to causing deformities in fungal cells, changing the permeability of the cell membrane, leaking its contents to the outside, and inhibiting the formation and germination of sclerotia [8].

As for the effect of the biological resistance fungus T. koningiopsis, it was found that the effect of the chemical pesticide Beltanol on pathogenic fungi may be attributed to its ability to works on the chelating substances in mushrooms. [3] [2].

mycelium of this biological agent covered some of the mycelium of the pathogenic fungus, which indicates that there is some kind of overlap between the mycelium of the pathogenic fungus and the biological fungus, as many species of the genus Trichoderma, to which this biological agent belongs, are characterized by their hyphae having relatively small diameters, which enables them to wrap around the hyphae of the pathogenic fungi and form compressive structures. With the help of some of the degrading enzymes it secretes, such as Proteases, cellulases, and chitinases, it can decompose the walls of fungal cells, and then penetrate and parasitize them [17].

Table (7) Biological and chemical control of the fungus Chaetomium madrasense, which causes root rot and death of chrysanthemum seedlings in plastic pots.

N	The treatment	Percentage of infection	Percentage of injury severity
1	Correct comparison	0.0	0.0
2	*C.madrasense is a* solitary pathogenic fungus	100	55.66
3	*Beltanol*	0.0	0.0
4	*T. koningiopsis*	0.0	0.0
5	*C. madrasense* + *T. Koningiopsis*	21.33	15.33
6	C.madrasense + Beltanol	19.66	12.66
7	C.madrasense + Beltanol +*T. Koningiopsis*	12.33	5.66
L.S.D 0.05		0.482	1.215

Conclusion

The study showed that the fungus Chaetomium madrasense has a pathological effect in inhibiting the germination of chrysanthemum seeds and reducing seedling viability, This is reflected in the plant, The comparison results showed that both biological and chemical control contributed to reducing infection, but biological control emerged as safer for the environment.

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