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First record of *Curvularia hawaiiensis* and *Cladosporium sphaerospermum* as a causative agent of spotting diseases in oleander *Nerium oleander* l and control it bio control and chemically

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Abstract
This study was conducted in the Plant Protection Department /College of Agriculture/ Basra University for the season 2019-2020. This study aimed to study leaf spot disease in oleander plant. The results of the field survey showed the presence of leaf spot disease in all regions, It showed the results of isolating the fungi *C. hawaiiensis* and *C. sphaerospermum* from the leaves of the oleander plant, the results of the pathological ability test indicated that these two fungi were capable of causing leaf spot disease, the results of the pesticide test showed the growth of fungi *C. hawaiiensis* and *C. sphaerospermum*, Where it was found that the most effective pesticides in inhibiting *C. hawaiiensis* It is fungicide Topas Where the inhibition rate reached 82.15% at a concentration of 300 ppm, and the least fungicide is the fungicide Aliette Express That amounted to 11.17% in concentration With a concentration of 300 ppm either *C.sphaerospermum*, The more pesticides Inhibition is fungicide Topas That amounted to 85.29% with a concentration of 200 and 300 ppm and the lowest pesticides that inhibit the fungus *C. sphaerospermum* It is Proxanel 37.05% at a concentration of 100ppm The study showed that all the used fungicides affected the sporulation of the isolated fungi, and that the most effective pesticides on the fungus spores *C.sphaerospermum* It is fungicide and Ortiva and Difecor As the number of spores in it reached zero. The results of the contrast test indicated the presence of high antagonism among the fungi *Trichoderma koningi*, and pathogenic fungi *Curvularia hawaiiensis* and *Cladosporium sphaerospermum*, where was the *T.koningii* inhibiting the growth of pathogenic fungi, as it reached the degree of antagonism (1) with both fungi, The results showed that the biological fungus had a positive role in increasing the peroxidase enzyme, as it gave the highest treatment of the biological fungus *Tricho +Curvu+ Clado*. Which amounted to 0.0976 units / g-wet weight compared to the comparison treatment, as the average peroxidase enzyme was 0.0128 units / g-wet weight. The results of the Symbiotic effect of the pesticide and the biological fungus on the severity of infection with the fungi that because the oleander spotted disease showed that the treatment with the pesticide, as well as the biological fungus, reduced the severity of the disease.

Keywords: *Curvularia Hawaiiensis, Cladosporium Sphaerospermum, Nerium oleander,*

Introduction
The origin of the oleander plant is the Mediterranean and is widely cultivated as an ornamental plant in tropical, subtropical and temperate regions for its abundant long-lasting flowers (Kingsbury, 1964; Hardin and Arena, 1974) [34, 20]. The oleander plant, Nerium oleander L, belongs to the family Apocynaceae, It is an evergreen shrub that spreads in all governorates of Iraq as decorative shrubs in gardens, parks and public places (Al-Shahat, 1988) [2]. The oleander has been used to decorate public streets and fences of gardens and parks in many countries (Huxley, 1992) [25]. Since 1979, the production of ornamental plants began to deteriorate due to outbreaks of diseases, including bacterial blight, wilt disease, and stain diseases caused by fungi, which had the most effect (Daughtrey and Benson, 2005) [12]. The oleander plant is exposed to many fungal diseases, including leaf spot disease, which is caused by many fungi, one of the most important of these fungi *Curvularia* sp. That causes important diseases on ornamental plants and other crops (Singh *et al*, 1997; Lin and Wan, 2006) [52, 37]. The fungus *Cladosporium* spp causes leaf spot disease on many plants (Soytong, 2014) [56]. *Cladosporium cladosporioides* causes leaf spot disease, secretes mycotoxins, Leaf spot disease, and secrete mycotoxins (Sreedevi *et al*., 2011) [57]. *Curvularia hawaiiensis* Leaf spot disease on pearl millet has caused great losses in recent years (Khatal *et al*, 2019) [33]. Chemical control is one of the most reliable ways to control various plant diseases in emergencies (Hanuman *et al*., 2014) [19]. Because of the increase in the cultivation of oleander in the province of Basra as a plant resistant to drought and salinity, the incidence of stain fungi increased, especially in places of plant breeding, as well as in places of plant cultivation in relatively saline areas. Stain diseases that affect oleander and the possibility of combating them chemically and biologically.

Materials and Methods
Study of symptoms
The infected leaves were collected separately in polythene bags and carried in laboratory and symptoms were studied with the help of microscope.

Fungal isolation and purification
Three 2 mm pieces samples of oleander leaves that showed symptoms, dark brown to black leaf spots were used to get fungal isolates. The pieces were surface sterilized in bleach (1% available chlorine) for 5 minutes, and washed twice in sterile water for 5 minutes. Then, the pieces were dried by placing them on sterile paper towel. Subsequently, tissue pieces were transferred onto quarter-strength potato dextrose agar (PDA), at rate of five pieces per plate, with three dishes per sample, the plates were incubated at a

temperature of 25 ± 2 C, and after 5 days the fungi were purified MEA. Then it was incubated in the incubator and afterwards it was diagnosed by observing its phenotypic and microscopic characteristics using a combined microscope Optika B-180 and depending on the classification keys (Ellis, 1976) [16] (Sivanisan, 1987) [55] (Bensch *et al*, 2012) [8].

Pathogenicity test

Took models from healthy oleander seedlings planted in plastic pots and divided the plants into two parts: a wound section and a section left without wound, then the spore suspension was taken concentrated 10^5 spore/ml each fungus has a pathogen *C. hawaiiensis* and C. *cladosporioides* the concentration of spores was controlled using a counting slide Haemocytometer It was sprayed by a manual sprayer with a volume of 2 liters on the leaves of the plant inside a plastic house in which moisture is suitable for plants and by three plants per fungi. The comparison treatment was only sprayed with sterile distilled water. Symptoms were observed on plants a month after infection, and then the severity of the infection was calculated according to measures consisting of five degrees. The experiment was carried out with three replications for each isolate.

Table 1

Rating Scale	number of spots
0	No thing
1	1-3
2	4-6
3	6-8
4	lower leaves die

Disease evaluation parameters the infection was identified on basis of symptoms present in the leaves. Thereafter, disease incidence was calculated as the number of infested plants showing symptoms out of total numbers of mango plants observed.

The percent disease intensity (PDI) was calculated using the formula developed by McKinney (1923) is given below: +

$$Occurrence = \frac{Sample\ Plants\ Infected}{1Total\ No.of\ Sample!} \times 100$$

$$Intensity\ = \frac{No.of\ Leaves\ or\ Units\ Infected}{Total\ No.of\ Leaves\ or\ Units\ of\ Infection} \times 100$$

The effect of different concentrations of fungicides on the growth of pathogenic fungi

In this experiment, six pesticides were used they are Topas, Ortiva, Difecor, Aliette Express and Proxanel. prepare a PDA medium and sterilize it in the steam autoclave as in the previous method, and after sterilization, it was left to cool until its temperature drops to pre-solidification, then distributed into glass jugs of 250 ml at a rate of 75 ml per beaker, then a basic solution was prepared at a concentration of 1000 ppm of each pesticide and transferred A certain amount of the base solution to the flasks containing the culture medium to obtain the concentration, 100, 200 and 300 parts per million of each pesticide.

The flasks to which pesticides are added shaken well for homogenizing the distribution of the pesticide with the medium.

The medium containing the pesticides was poured into a 9 cm diameter Petri dish, after which the dishes were inoculated with 0.5 cm diameter tablets of isolated and grown fungi on a sterile PDA medium. as for the comparison treatment, it included isolates of fungi in an agricultural medium free of pesticides, all dishes were incubated at a temperature of 25 + 2 C for a period of 7 days after which according to the growth rate of the fungus by taking the average of two perpendicular drops passing through the center of the colony from the back and the percentage of growth retardation was calculated according to the modified About equation presented in Shaban and Al-Mallah (1993) [50].

$$Inhibition\% = \frac{\text{Diameter growth rate in comparison} - \text{rate of diameter growth in the equation}}{\text{Diameter growth rate in comparison}} \times 100$$

Effect of fungicides on bacteriostatic of pathogenic fungi

Pesticides added Topas, Difecor, Aliette, Express, Proxanel and Ortiva to the medium (PDA) at a concentration of 100 ppm per pesticide, with a comparable treatment without pesticide after that, the media was poured into sterile petri dishes with a diameter of 9 cm and after hardening of the media, the dishes were inoculated with a 0.5 cm drop of isolated fungi for each dish 0.5 cm of fungus growing on a medium containing pesticides and put in a glass tube containing 4.5 ml sterile distilled water Shake the tube well for five minutes to remove the spores of the fungus then take 1 ml of the suspension and add 9 ml of sterile distilled water and repeat the process several times to obtain dilution 10^{-3} the Haemocytometer slide was used to calculate the number of spores in each ml. Then the number of colonies produced was calculated and the number of spores in each ml were extracted from the following equation:

Number of spores / ml = number of colonies x dilution mold.

This experiment was carried out with three replications and using a fully randomized design, the double implantation method of fungi was used to test the ability of the fungus to be biocompatible with the pathogenic fungi, by dividing a petri dish containing a sterile PDA medium into two equal parts and then inoculating the first section at a distance of 1 cm from the edge of the plate with a disc of 0.5 cm diameter from the fungus colony *Trichoderma koningi* Seven days old, the second section was inoculated at a distance of 1 cm from the edge of the plate with a 0.5 cm diameter disc from the pathogen colony with a control treatment inoculated with a 0.5 cm diameter disc of the pathogen alone and with three replications for each treatment and the control treatment, then the dishes were incubated at a temperature 25±2 C° The degree of contrast was calculated after the growth in the control treatment reached the edge of the plate according to (Bell *et al*,1982). It consists of five degrees, as follows:

Table 2

Degree of contrast	Description of the contrast
1	anti-fungus covers the entire plate
2	including the pathogen, anti-fungus covers 2/3 of the plate
3	anti-fungus covers half of the plate,
4	pathogenic fungus covers two thirds of the plate
5	pathogenic fungus covers the whole plate

The antifungal is effective if the degree of antagonism is one or two.

Synergistic effect between the fungus of biological resistance and the fungicide Topaz in infection of the oleander plant with the pathogen

This experiment was carried out at the Agricultural Research Station at the College of Agriculture inside the greenhouse. The experiment land was divided into three sectors, with a length of 9 m for each mole and an area of 72 m between a mole and another. *c. hawaiiensis* and *c. sphaerospermum* At a concentration of 10^5 spore / ml, adjust the concentration using the acne strip Hoemocytometer, The comparison treatment included spraying the plants with distilled water, and after three days, the plants were sprayed with the fungicide suspension *T. koningii*, and after two days, sprayed the plants with the fungicide Topas, after 7 weeks, the severity of the pathogenic fungi infection was measured according to the evidence mentioned in paragraph (3-5).

Determination of peroxidase enzyme in oleander leaves

The leaves of the oleander plant were taken and placed in polyethylene bags, each instructed according to his treatment, and placed in a box containing ice and transported to the laboratory. Then I took 150 mg soft weight from the leaves of the oleander plant, then washed with anion-free distilled water, then 2.5 ml of phosphate buffer was added to it potassium phosphate buffer at a concentration of 0.05 mol, consisting of dehydrogenase potassium phosphate (K_2PO_4) and potassium mono hydrogen phosphate K_2 with a pH of 6, the mixture was placed in a centrifuge at 12000 rpm for 20 minutes and then added to 250 mμ of Coaicol stain at a concentration of (0.5%) and hydrogen peroxide at a concentration of (0.3%) v / v and 2.5 of a phosphate buffer solution. The absorption was read directly in a Spectrophotometer at 470nm wavelength (Kim *et al*, 1988), by three iterations for each treatment, the enzymatic activity was estimated based on an enzymatic absorption unit per gram of soft weight, and the enzymatic activity was calculated according to the following equation:

Enzyme Activity (μmol/min ml) or (U/ml) = (Consumed Substrate) (μmol /ml) ×Total Reaction Volume (ml) / (Reaction time (min)) × (Enzyme volume (ml)).

Results and Discussion

Isolation and diagnosis of fungi causing oleander leaf spotted disease

A group of fungi was isolated from oleander leaves as shown in table using the culture medium PDA and MEA from oleander leaves that showed symptoms of spotted disease in different areas of Basra Governorate and the fungi are considered pathogens *C. hawaiiensis* and *C. sphaerospermum* One of the most important causes of plant leaf staining, including oleander, has been diagnosed based on the taxonomic characteristics that he listed Ellii (1971 and 1976) [15, 16], Sivanisan (1987) [55], Crous *et al* (2007), and Bensch (2012) [8].

Table 3: fungi isolated from oleander leaves that showed symptoms of spotting

Isolation areas	fungus
Shatt al-Arab District	*Chaetomium* sp *Curvularia* sp *Cladosporium* sp
Abi AL -Khasib district	*Curvularia* sp *Cladosporium* sp
Karma Ali	*Cladosporium* sp *Curvularia* sp *Chaetomium* sp
Al Khora	*Cladosporium* sp *Curvularia* sp

Test the pathogenicity of fungi that cause oleander leaves staining

The results of the pathogenicity test of different fungi isolates demonstrated the ability of the fungi *C. hawaiiensis* and *C. sphaerospermum* Induced infection with leaf spot disease on the oleander plant, as for the fungus *Chaetomium* sp no symptoms were recorded on the oleander plant, and the symptoms of infection were represented in the form of small-sized brown spots surrounded by a yellow halo, and over time, it expanded to include most of the leaf surface, and this was confirmed by Aslam and others (2019) [6], where he indicated that the symptoms of spotting resulting from the fungus *Curvularia hawaiiensis* they are circular, purple-tilted spots, 0.7 to 1.0 cm in diameter, with gray centers surrounded by a yellow halo, Abd al-Qadir and Muhammad (1997) [1] and Al-Zayyat and others (2002) [3] who indicated that the spots that appeared on palm leaves are often shared by many fungi, including *Cladosporium*

Effect of different concentrations of some fungicides on the growth of *C. hawaiiensis* and *C. sphaerospermum*

The results of this experiment, Table (3), showed that there were significant differences in the percentage of inhibition of fungus *Curvularia hawaiiensis* The rate of inhibition of fungus growth was 76.27, 64.28, 58.49, 18.49 and 13.73% for the pesticides Topas, Difecor, Ortiva, Proxanel and Aliette Express, respectively, as it was noted that Topas was the most effective among other pesticides in inhibiting the fungus and the least effective pesticides in The growth of the fungus is Aliette Express, as it was found from the same table that the effect of pesticides on the growth of the fungus increases with the increase in the concentration used, as the percentage of the rate of inhibition of the growth of the fungus was 44.31, 44.47 and 49.96% for the concentrations of 100, 200 and 300 ppm, respectively.

The interaction between the pesticides and the concentration was significant, as The effect of the pesticide Aliette Express at a concentration of 100 parts per million more than the rest of the concentrations, while the effect of the pesticide Ortiva at a concentration of 100 parts per million was more than its effect at a concentration of 200 parts per million.

As for the results of the effect of the mentioned pesticide concentrations on C. sphaerospermum, they also indicated the presence of significant differences, as the rate of inhibition of fungus growth was 83.5, 75.6, 70.1, 66.3 and 39.9% for the pesticides Topas, Difecor, Ortiva, Aliette Express and Proxanel, respectively. Topas and the lowest

effect on the growth of the fungicide Proxanel, and also the effect of the pesticides increased by increasing the concentration, except for Aliette Express, its effect was at a concentration of 100 ppm more than the rest of the concentrations.

These results are consistent with what was mentioned by Fayyad and Manea (2008) [18], as Topas and Ortiva inhibit the growth of the fungus *Cladosporium herbarum* that causes spotting on palm leaves. Singh and others (2014) [51] indicated that Topas and Difecor affect Curvularia sp, and Topas works to inhibit the growth of the fungus Cladosporium herbarum (Mehboob *et al*., 2015) [38]. It also agreed with the results of the study of Al-Abboudi (2019) that all laboratory concentrations of Topas showed an inhibition ratio for both the fungi *Cladosporium oxysporium* and Curvularia lunata. It was also mentioned that Difecor and Ortiva inhibited the growth of C. Sumangala *et al*. (2008) showed in a laboratory experiment to evaluate four systemic fungicides that Difecor works to inhibit the fungal growth of *Curvularia lunata*. This result was agreed with Cuina *et al*. (2014) [11] as well as with Karmakar and others (2016) [30]. lunata with seven pesticides, including Ortiva, which inhibited the growth of the fungus (Persaud *et al*., 2020) [45] and agreed with Waris *et al*. (2018) [61].

The decrease in the percentage of Aliette Express in inhibiting Curvularia hawaiiensis was in agreement with the results of Brown *et al*. (2004) [10], who indicated that there was a clear failure in the fungicides that contain aluminum phostyl (the active ingredient of the pesticide Aliette Express). For leaf spot disease on leeks as well as the fungus *Cladosporium allii-cepae* on onions (Jordan *et al*., 1990) [29]. As for the results of Aliette Express on *Cladosporium sphaerospermum* and its high inhibition rate, Saleem *et al*. (2012) [48] agreed.

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Aliette inhibited the fungus *Cladosporium* sp in an experiment that tested eight pesticides on fungi that infect corn seeds. *Cladosporium cladosporoides* and *Curvularia pallescens* were also inhibited in a study of chemical activity in controlling hot villa seed fungi (Sitara and Hassan, 2011) [54].

As for the pesticide Proxanel, it was the least inhibiting fungicide studied, and it agreed with the results of Mehboob and others (2015) [38], as it was the least inhibition of the fungus Cladosporium herbarum and that the reason for the fungi to be less affected by the pesticide or not affected by the pesticide in the first place is because of the ability of the fungi to break down the pesticide particles (Bodyn, 1996) [9].

Table 4: The effect of different concentrations of some fungicides on the growth of *C. hawaiiensis* and *C. sphaerospermum*

Pesticides	**% To inhibit the growth of fungus**							
	Concentration (ppm)							
fungi	*Curvularia hawaiiensis*				*Cladosporium sphaerospermum*			
	100	200	300	Average effect of pesticides	100	200	300	Average effect of pesticides
Difecor	55.88	62.86	74.11	64.28	75.5	76.3	75.1	75.6
Aliette Express	18.23	11.77	11.17	13.73	72.2	70.6	56.3	66.3
Topas	73.13	80.0	80.0	85.3	80.0	85.3	85.3	83.5
Ortiva	58.23	56.86	60.39	58.49	66.3	68.0	76.1	70.1
Proxanel	16.07	17.45	21.96	18.49	37.1	44.5	38.2	39.9
Average effect of concentrations	44.31	44.47	49.96	64.28	66.2	68.9	66.2	75.6
R.S.L.D 0.05	concentration = 4.313, pesticides = 5.568, overlap = 18.02							

Effect of fungicides on bacteremia of *C. hawaiiensis* and *C. sphaerospermum*

Table (4) showed that all the used fungicides had an effect on the bacterium of *Curvularia hawaiiensis*, compared to the comparison treatment of 7.33 x 310 spore / ml. In addition, statistically significant differences were found between some of the pesticides used, as the average number of spores per 1 ml of mushroom suspension was 2.33, 4.33, 0, 1.66 and 2.33 x 310 spore / ml for each of the pesticides Difecor, Aliette Express, Topas, Ortiva, and Proxanel Where it was found that Topas is the most effective fungicide bacterium. As for *Cladosporium sphaerospermum* Also, all used fungicides affected it according to the comparison treatment reached 24.66 x 310 spore / ml also, significant differences were found between some pesticides used, as the average number of spores per 1 ml of mushroom suspension 0, 1.66, 0, 0, 20.66 x 3^{10} spore / ml for Difecor, Aliette Express, Topas and Proxanel pesticides. Topas, Ortiva, and Difecor are the most effective in inhibiting *C. sphaerospermum* in a previous study; Kenyon *et al*. (1997) [31, 32] indicated that Topas reduced bacteremia *Erysiphe sp*, which causes powdery mildew. Likewise, the effect of Ortiva on the bacterium of *Plasmopara viticola* (Wong and Wilcox, 2001) [62] and between Anesiadis and others (2003) [4].

Ortiva and Difecor affect the bacterium of *Cercospora beticola* and *Erysiphe betae*. In general, Proxanel reduces fungi bacteremia to a minimum (Mensin *et al*., 2013) [39] by reducing the bacterium Phytophthora infestans (Johnson *et al*. 2000) [28].

Panicker and Gangadharan (1999) reported that treatment with Aliette Express reduced or prevented the bacterium of *Peronosclerospora sorghi*, which causes downy mildew in corn.

Table 5: Effect of fungicides on the bacterium Curvularia hawaiiensis and C. sphaerospermum

fungi	fungi spores rate Curvularia hawaiiensis Per / 1 ml (3^{10})	fungi spores rate C. sphaerospermum Per / 1 ml (3^{10})
Control	7.33	24.66
Difecor	2.33	0
Aliette Express	4.33	1.66
Topas	0	0
Ortiva	1.66	0
Proxanel	2.33	20.66
R.L.S.D 0.05	1.9	3.7

Test of the antifungal potential of *T. koningii* with pathogenic fungi

The results of this test showed the presence of high antagonism between *Trichoderma koningii* and the pathogen *Curvularia hawaiiensis* and *Cladosporium sphaerospermum*, where *T.koningii* inhibited the growth of pathogenic fungi in a clear way, as it reached the degree of antagonism (1). Between *T. koningii* and *Curvularia hawaiiensis* and (1) degree between *T. koningii* and *Cladosporium sphaerospermum* on the Bell scale. This is in agreement with Adejumo and Orole (2009) [42] that *T. koningii* has achieved a high antibody capacity against *Cladosporium herbarum*, as *T.koningii* can be used as a fungicide in the control of corn wilt and seedling blight. Nashwa, Sallam, and others (2008) [41] indicated that T.koningii showed strong hostility against *Cladosporium* sp and agreed with the findings of Leelavathi and others (2014) [36]. *T. koningii* was found to act as an antagonist against *Curvularia* sp (Tapwal *et al*., 2015) [60]. The anti-effect of *Trichokonins* taken from *T. koningii* on *C.lunata* was selected to inhibit its growth (Xiao-yan *et al*., 2006) [63]. This result is consistent with the findings. The mechanism of Tapwal *et al*. (2015) [60]. The antibacterial ability of *T. koningii* is due to the fact that it works to compete with the parasite, acts as an antibiotic, and secretes enzymes that protect the cell wall of the plant and promote plant growth and thus induce resistance (Howell, 2003 and Benitez *et al*., 2004) [23, 7]. The efficacy of Trichoderma in biological control or opposition to pathogenic fungi is directly proportional to the availability of iron, boron, copper, dissolved magnesium, nitrates, and trace amounts of phosphorous and pH (Duffy *et al*., 1997) [13].

Synergistic effect between the fungus of biological resistance and the insecticide Topaz in infection of the oleander plant with the pathogen

The results showed significant statistical differences in the severity of infection, Table ( ), where the interaction treatments containing the pesticide Topas and the biological fungus T.k were distinguished by reducing the severity of infection with leaf spot disease on the oleander plant compared to the treatment of the pathogen alone, where the treatment of the pathogen Curv reached 49.55 and treatment with the pathogen Clado fungus. 56.3 The interaction coefficients for the pesticide and the biological fungus Topas + Curv + Tricho were 29.62 and Topas + Clado + Tricho reached 33.38.

The treatments containing the pesticide and the pathogen exceeded all of the treatments, with the Topas + Curv treatment. 19.76 And Tobas + Clado 19.70 treatment. These results are consistent with many studies where Topas was applied in grape fields and impeded the development of Uncinula tiecator (Reuveni and Reuveni, 1995) [47]. In a field experiment, Topas was used in apple and pear fields suffering from apple and pear scab disease caused by the fungus. Venturia inaequalis and V. pirina and its effectiveness as a protective material against scabies has been proven when applied (Percival *et al*., 2009) [43]. Anon (2008) [5] confirmed that Topas' action in reducing the severity of apple and pear scabs is to increase the chlorophyll content. The effectiveness of this pesticide has been proven by other researchers (Kenyon and others, 1997, Percival and Boyle, 2005 and Schnabel and Parisi, 1997) [31, 32, 44, 49]. and in a study aimed at applying biological organisms, including *T.harzianum*, to control disease. The oil palm leaf spot produced by the fungus *Curvularia oryzae* reduced the incidence of this disease because treatment with the selected isolate of Trichoderma enhanced the activity of the enzymes Phenylalanine ammonia-Lyase (PAL), Peroxidase (POD), and Polyphenol oxidase (PPO) (Sunpapao *et al*., 2008). Elad (2000) [14] stated that *T.harzianum* is one of the biological control agents of the fungus *Botrytis cinerea*, which causes gray rot disease in greenhouses and grape crops that are infected with the fungus *Sclerotinia sclerotioum* that causes white rot in greenhouses and fields, and it was also applied to *Cladosporium fulvum*, which causes leaf rot in tomatoes. T.h was used as a biological control agent in onion fields to reduce the severity of onion infection with the purple spot disease caused by *Alternaria porri* and to promote growth in sensitive onions. Several methods of plant treatment were evaluated, including seed treatment, dipping seedlings, and three sprays, all of which resulted**.** To suppress disease and promote growth (Prakasam and Sharma, 2012) [46].

Table 6: the synergistic effect between the fungus bio-resistance and the pesticide Topaz in infecting oleander with pathogenic fungi

Treatments	severity of the injury
Clado.	56.3
Curv.	49.55
Tricho. +Curv.	38.89
Tricho. +Clado.	41.32
Clado+ Topas	19.70
Topas +Curv	19.76
Topas +Curv. +Tricho.	29.62
clado +Tricho+ Topas	33.38
Con.	11.76
LSD 0.05%	7.3

Determination of peroxidase enzyme in oleander leaves (unit / g fresh weight)

The results, Table (6), showed that the biological fungus had a positive role in increasing the peroxidase enzyme, as it gave the highest treatment of the biological fungus Tricho + Curvu + Clado, which reached 0.0976 units / g wet weight, followed by the Tricho + Clado treatment, which reached 0.0896 units / g wet weight, then the treatment. Tricho, which amounted to 0.0687 units / g wet weight, followed by Tricho + Clado unit / g wet weight, then the treatment Tricho + Topas unit / g wet weight, which differed significantly from other treatments. As for the pesticide treatment, it differed from other treatments with significant differences as well, as the treatment with pesticide reached Topas 0.0652 units / g Wet weight Several studies have

indicated the ability of biological fungi to induce resistance in plants against live pathogens by increasing the activity of the peroxidase enzyme in the plant. Research on the mechanisms used by *T.virens* to control *Rhizoctonia solani* that infects cotton has shown that when treated with seeds with *T.virens*, the defense response is stimulated by increasing the activity of the peroxidase enzyme (Howell *et al*., 2000) [24]. When treating onions with five species of Trichoderma, *T.koningii*, *T.viride*, *T.virens*, and T.harzianum, the plant showed resistance to the white onion rot disease caused by *Sclerotium cepivorum* by increasing the activity of Peroxidase, Polyphenol oxidase and Chitinase (Elshawy *et al*., 2017) [17].

The treatment of sunflower with *T.harzianum* and *Glomus* spp. Increased the activity of peroxidase enzyme and resistance to *R.solani* (Sirin, 2011) [53]. As for the increase in enzyme activity when treated with Topas, I agreed with many results, as the effect of Penconazole, which is the active substance of Topas, on the physiological changes and activities of several antioxidant enzymes in the catnip plant under the pressure of water shortage and the external application of this pesticide increased some growth parameters. Among them is the enzyme peroxidase (Hassanpour *et al*., 2013) [21] and between Jaleel and others (2006, 2007a) [26, 27].

Topaz is a triazole group of fungicides that have plant growth regulating properties and have the ability to boost antioxidants. Hibar and others (2007) [22`] stated that the mechanism of action of the peroxidase enzyme against pathogenic fungi includes in the structural defenses to strengthen the cell walls such as building lignin and the interaction of the enzyme with cell wall proteins and the formation of cross-links as it increases the wall stiffness, as well as the peroxidase enzyme works with hydrogen peroxide in breaking down the pathogen enzymes.

Table 7: Determination of the peroxidase enzyme in the leaves of the oleander plant (unit / g fresh weight)

Treatments	enzyme unit / g weight
Clado.	0.0190
Curv.	0.0165
Topas	0.0652
Topas +Curv.	0.0155
Topas+Clado.	0.0122
Curvularia+clado.	0.0244
Tricho.	0.0687
Tricho. +Curv.	0.0661
Tricho. +Clado.	0.0896
Tricho. +Topas.	0.0630
Topas +Curv. +Clado.	0.0321
Tricho. +Curv. +Clado.	0.0976
Topas +Curv. +clado. +Tricho.	0.0199
Con.	0.0128
LSD	0.0117

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