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المجلة العراقية لبحوث السوق وحماية المستهلك

INTEGRATED CONTROL OF ROOT ROT AND WILT DISEASE ON *CATHARANTHUS ROSEUS* USING BIOLOGICAL AND CHEMICAL CONTROL

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ABSTRACT

This study was conducted at the University of Baghdad, College of Agricultural Engineering Sciences/Department of Plant Protection for the period 2021-2022, with the aim of isolating and diagnosing the pathogens that cause root rot and wilt disease on *Catharanthus roseus* in different areas of Baghdad, and conducting an integrated control of the pathogen using biological and chemical control. The results of isolation and identification the presence of 4 types of fungi accompanying plants: *Fusarium oxysporum*, *F. solani*, *F. equiseti* and *Rhizoctonia solani* in different nurseries in Baghdad regions, and the most frequent species were the species *F. solani*, *F. equiseta* (FeL1), The results of the antagonism test showed that the commercial preparation of *Trichoderma harzianum* had a high antagonistic ability against FeL1 on the PDA medium. The results also showed that the use of Beltanol at concentrations (50, 100, 500, 1000, 1500 and 2000) mg/L led to a 100% inhibition of the growth FeL1 for concentrations, compared to the control treatment without fungicide on PDA in which the inhibition rate was 0.00%, and the use of Beltanol at a concentration of (50) mg/L did not have a significant effect on the *T. harzianum* with significant differences from the rest of the concentrations used in the test, as well as the results of the effect of the efficiency of the *T. harzianum* and the Beltanol alone and their interactions in combating the disease under the conditions of the greenhouse and the nursery showed that All treatments led to a decrease in the rate and severity of infection with the pathogenic *F. equiseti* and an increase in the fresh and dry weight of the vegetative and root total of seedlings of the dwarf variety.

Keywords: Catharanthus roseus, Fusarium spp., Trichoderma harzianum, Beltanol

المكافحة المتكاملة لمرض تعفن الجذور والذبول على *Catharanthus roseus* باستعمال المكافحة الاحيائية والكيميائية

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الخلاصة

اجريت هذه الدراسة في جامعة بغداد كلية علوم الهندسة الزراعية/ قسم وقاية النبات للفترة 2021-2022 بهدف عزل وتشخيص المسببات المرضية المسببة لمرض تعفن الجذور والذبول على نبات عين البزون *Catharanthus roseus* في مناطق مختلفة من محافظة بغداد وإجراء مكافحة متكاملة للممرض باستعمال المكافحة الأحيائية والكيميائية، بينت نتائج العزل والتشخيص وجود 4 أنواع من الفطريات المرافقة للنباتات هي *Fusarium oxysporum*، *F. solani*، *F. equiseti* و *Rhizoctonia solani* تباينت في الظهور باختلاف مشاتل مناطق بغداد وكان أكثر الأنواع تكراراً هي الأنواع *F. oxysporum*، *F. solani*، *F. equiseti* وبينت نتائج اختبار التضاد أن للمستحضر التجاري للفطر *Trichoderma harzianum* قدرة تضادية عالية ضد العزلة الممرضة *F. equiseti* (FeL1) على الوسط الغذائي PDA، كما أظهرت النتائج ان استعمال مبيد Beltanol بالتراكيز (50 ، 100، 500 ، 1000 ، 1500 و 2000) ملغم / لتر أدى إلى تثبيط نمو العزلة الممرضة (FeL1) بنسبة 100% لجميع التراكيز قياساً بمعاملة المقارنة من دون المبيد إلى

[*] البحث مستل من رسالة ماجستير للباحث الاول.

الوسط الزرعى التي كانت نسبة التثبيط فيها 0.00%، وان استعمال Beltanol بالتركيز (50) ملغم / لتر لم يكن له تأثير كبير على *T. harzianum* وبفروق معنوية عن باقي التراكيز المستعملة، كذلك أظهرت نتائج تأثير كفاءة *T. harzianum* والمبيد Beltanol منفرداً وتداخلاتهما في مكافحة المرض تحت ظروف البيت البلاستيكي والمشتل أن جميع المعاملات قد ادت الى خفض نسبة وشدة الإصابة بالفطر الممرض *F. equiseti* وزيادة في الوزن الطري والجاف للمجموعين الخضري والجذري للشتلات للصنف القزمي.

الكلمات المفتاحية: *Catharanthus roseus*، *Fusarium spp*، *Trichoderma harzianum*، Beltanol.

INTRODUCTION

Natural ornamental plants are one of the most beautiful elements of interior design due to their beauty and giving the place spirit and life. Therefore, many people want to have these plants in their homes and offices to relieve the pressures of daily life (**Safi, 2015**). The *Catharanthus roseus* plant is one of the groups of ornamental plants that plays an important role in the practical coordination of public and private gardens. It is a perennial herbaceous perennial evergreen plant. It has abundant branches, the leaves are simple, opposite, often seated, the flowers are large and of many colors, with a single eye shape, that's why it's called the cat's eye. It is cultivated in Iraq for decorative purposes **(Al-Ammari *et al.*, 2018)**. Most ornamental plants, like other plants, are exposed to many pests and diseases that affect their growth **(Abu Zahra & Al-Qasim, 2015)**. Including diseases caused by fungi endemic to the soil, such as diseases of seedling damping off, root rot and crown area. These diseases are caused by a number of fungi of different genera, including *Rhizoctonia*, *Fusarium*, *Sclerotium*, *Verticillium*, *Phytophthora*, *Pythium*, **(Al-Haidari & Mahdi, 2015)**, and diseases that affect the vegetative system, such as leaf spot and blight, caused by the genera *Colletotrichum*, *Cercospora*, *Alternaria*, *Bipolaris*, *Ascochy*, and Botrytis blight or Mold Gray caused by the fungus *Botrytis* spp. or the pathogen may be a bacterial, *Erwinia*, *Xanthomonas*, and powdery mildew caused by the fungus *Oidium* spp. Downy mildew is caused by *Peronospora sparsa*, and black spot disease is called leaf spot, and this disease is caused by the fungi *Marssonina rosae*, *Asteroma rosae*, *Actinonema rosae*, *Marsonia rosae* **(Abu Zahra & Al-Qasim, 2015)**. A number of researchers also indicated that the species *F. semitectum, F. solani, and F. oxysporum* causes various diseases including seed rot, seedling damping of, root rot and wilting of plants of the oleander family **(Nejat *et al.*, 2015)**. Wilt is one of the most important and widespread fungal diseases caused by species of the genus *Fusarium*, especially *F. oxysporum* and *F. solani.* It is the most common fungal species that has been found to be associated with wilt, which limits the success of plant cultivation as it leads to significant losses in yield. For many ornamental plants, including the *C. roseus*, it may reach a percentage of 50-85% **(Hami *et al.*, 2021)**.

Integrated control has gained increasing attention by a large number of specialists in the field of plant disease control, as the introduction of biological with chemical control has significantly reduced the intensity of pathogens **(Al-Amiri & Al-Bdour, 2016)**. Biological factors and chemical fungicide s have shown their role in protecting the seeds and seedlings of watermelon from infection with pathogenic fungi. **Muhammad (2008)** pointed out the importance of using the fungus *T. harzianum* and the chemical fungicide Beltanol in protecting palm saplings at the age of 6 months from infection with some types of fungi *Fusarium* spp., which are *F. solani*, *F. oxysporum* and *F. graminearum*, which cause palm wilt disease. The biological fungus and the chemical fungicide succeeded in protecting the palm saplings from the aforementioned pathogenic fungi and reducing the severity of the infection with pathogenic fungi. Lately, it has been observed the emergence of disease symptoms on the plants of Ain al-Bazoon in some nurseries as well as home gardens, represented by a state of rapid wilting that appears on the vegetative group on the plant seedlings and on large plants in the flowering

stage. Lately, it has been observed the emergence of disease symptoms on the plants of *C. roseus* in some nurseries as well as home gardens, represented by a state of rapid wilting that appears on the vegetative parts on the plant seedlings and on large plants in the flowering stage. This is accompanied by the rotting of the root system of the plant, and because the plant is of economic importance as an ornamental plant and a medicinal plant, the study aimed to investigate the infection of the disease in some nurseries of Baghdad governorate, isolate the pathogen and control it chemically and biologically.

MATERIALS AND METHODS

Sample collection

Samples of infected *Catharanthus roseus* plants that show symptoms of wilting and root rot, represented by pallor of lower leaves, yellowing and drooping of large leaves as a result of curvature of the petioles of leaves and wilting of plants completely, were collected from a number of important nurseries and some home gardens in Baghdad governorate (Al-Grayat, Al-Sidiya, Al-Khadra, Al-Dora and Al-Ghazaliya) for the 2021 season. The samples were placed in polyethylene bags, and the date of sampling and collection site was recorded on them. While the seeds were obtained from commercial sales offices of the type (dwarf). The samples were brought to the Plant Pathology Laboratory of the Plant Protection Department - College of Agricultural Engineering Sciences - University of Baghdad - Al- Jadriya for the purpose of isolation and diagnosis.

Isolation and diagnosis

The infected plants were washed with water to remove the dust and dirt stuck to them, and the roots and stems were cut into pieces (0.5-1) cm long, then superficially sterilized with sodium hypochlorite solution (1% free chlorine) for two minutes. The pieces were transferred to 9 cm diameter Petri dishes containing sterilized PDA (Potato Dextrose Agar) sterilized with an autoclave, at 121°C and a pressure of 5.1 kg/cm^2 for 15 min. Then the nutrient medium was cooled and the antibiotic Tetracycline was added at a concentration of 200 mg/L to prevent bacterial growth. Four plant pieces/plate were grown and the plates were incubated in the incubator at 25 ± 2 °C, and the growth of fungi was monitored for each plate after 3 d. After the appearance of fungal colonies around the infected parts, the fungus was purified by taking part of the edge of the colony and replanting it in other dishes and incubated for 5 d. Microscopic examination was carried out to determine the type of the associated fungi, and then the common genera was diagnosed under the small power of the compound microscope based on the spores and the sexual and asexual structures that the fungus formed.

Then the fungi were identified phenotypically to the level of genus and type by an Assist. Professor. Bushra Saber Abdul-Sada al-Maliki, using the approved taxonomic keys **(Lesile & Summerell, 2006)**. The percentage of the appearance and frequency of the fungus in the samples was calculated according to the following equation:

$$\text{Frequency of fungi in the sample (\%)} = \frac{\text{Number of pllant parts infected with the fungus}}{\text{The total number of pieces used for each sample}} \times 100$$

Purification and preservation of isolates of fungi

Six isolates of pathogenic fungi, three isolates of *Fusarium equiseti*, one isolate of *F. oxysporum* and two isolates of each *F. solani*, were purified by single spore technique to obtain pure and homogeneous cultures of the fungi isolates, according method to **Scott's & Chakraborty (2010)**. The purified isolates were kept in test tubes containing loam soil

sterilized by the autoclave and with three replicates, and then it was placed in the refrigerator at a temperature of 4 ° C until the subsequent tests are performed.

Pathological ability test on *Catharanthus roseus* plants in pots

The isolates were prepared: FsL1, FsL2, FsL3, FsL4, FsL5, FsL6, FsL7, FsL8, FsL9, FsL10, FeL1, FeL2, FeL3, ------ etc. Fungal isolates were grown on seeds of local millet *Panicum miliaceum* after being washed and sterilized in glass flasks by autoclave device at 121°C and a pressure of 1.5 kg/cm^2 for 15 min for two successive times. Then the flasks were inoculated with five discs with a diameter of 0.5 cm taken from the edge of the fungal cultures grown on the PDA culture medium at the age of 7 d and each separately.

The flasks were incubated at 25±2 °C for two weeks with shaking from time to time to distribute the inoculum to all seeds. Then, loam soil and peat moss were sterilized in a ratio of 2: 1 (weight: weight) with the autoclave and re-sterilized twice, then the soil was distributed in plastic pots of 1 kg, and the inoculum of each of the isolates was added to the potting soil at a ratio of 2% (weight/ weight) and each treatment was repeated four times with control treatment (sterilized seeds only added). Then the pots were covered with polyethylene bags and the bags were punctured for ventilation, and after 3 d, *Catharanthus roseus* plants were planted for the variety (dwarf). The infection rate was calculated 30 d after planting and the severity of infection was calculated according to the following scale:

0 = healthy plant, 1 = 25% of the roots are infected (brown contamination), 2 = 50% of the roots are infected (50% contamination without infecting the crown area), 3 = 75% of the roots are infected (75% contamination with infection of the crown area), 4 = 100% of the roots are infected (dead plant).

The severity of the injury was calculated according to equation **Mickenny's (1923)**.

Test the efficiency of the biological agent *Trichoderma harzianum* in inhibiting the growth of the pathogen in vitro

The antagonistic ability between the biological agent *T. harzianum* and the pathogen that proved to be pathogenic and more virulent was tested by using the double culture method by preparing sterile PDA nutrient medium in Petri dishes with a diameter of 9 cm. The petri dish divided into two equal parts with an imaginary line and then inoculate the center of the first section with a 0.5 cm disc of biological agent colony *T. harzianum* at the age of 5 d, while the second section was inoculated with a similar disc of pathogenic fungus growth for five days with a control treatment to inoculated with a disc of pathogenic fungi only and with three replicates for each treatment. The plates were then incubated at 25 ± 2 °C, and after 7 d, the antagonism was estimated according scale consisting of 5 degrees.

Test the effect of different concentrations of the chemical fungicide Beltanol on the growth of the biological factor and the pathogen

Concentrations of (50, 100, 500, 1000, 1500 and 2000) mg/L were tested, calculated on the basis of the active substance of the fungicide Beltanol, the active substance 50% Chinosol (8-Hydroxy quinoline sulfate) produced by the Spanish company Probelte. PDA nutrient medium was prepared and cooled to 45°C. Then each concentration was added to the medium separately. Then pour the medium into sterile dishes with a diameter of 9 cm, and with 4 replicates for each concentration, after solidification, each plate was inoculated in its center with a disk of 5 mm diameter from the edge of the plate of the pathogenic fungus and the biological agent grown on the nutrient medium PDA at the age of 7 d. While the control dishes,

they contained the nutrient medium PDA without fungicide s, inoculated with the pathogenic fungus and the biological agent, and with four replicates. The dishes were placed in the incubator at 25±2°C, after 5-7 d, the results were recorded by calculating the average measurement of two perpendicular diameters from each colony. The percentage of pathogenic fungus inhibition and biological factor was calculated by following equation:

$$\text{Inhibition (\%)} = \frac{\text{Average diameter of control colony - Average diameter of treatment colony}}{\text{Average diameter of control colony}} \times 100$$

Effect of the efficiency of the biological agent *T. harzianum* and the chemical fungicide Beltanol in controlling disease under greenhouse conditions

The experiment was carried out in one of the greenhouses of the Plant Protection Department during the growing season 2021-2022, following the of randomized complete block design (RCBD), using the biological agent that proved to be efficient in reducing the growth of the pathogen in the previous experiment. The seedlings of the plants, about 30 d old, were transferred from one of the nurseries in Baghdad Governorate (Al-Grayat) to plastic pots with a capacity of 1 kg, containing a mixture of soil and peat moss at a ratio of 1:2 and sterilized by an autoclave at 121 °C and a pressure of 1.5 kg/ cm^2 for a period of 20 min for two consecutive times separated by 1 day. There was 2 plants/ pot of the dwarf variety that were previously contaminated with the inoculum of a kind of pathogenic fungi, *F. equiseti*. A type of pathogenic fungi was selected, which was the most pathogenic. As a hole/ pot was made and 10 g of polluted millet seeds were added to it according to the treatment and then moistened with water and left for 3 d, then the seedlings of were transferred by two plants/pot of the dwarf variety and five replications for each treatment. Then the experiment was treated with the following treatments:

1) Control 1 untreated millet seeds
2) Control 2 millet seeds contaminated with the pathogen
3) Pathogen + chemical fungicide Beltanol
4) Pathogen + Biological preparation *T. harzianum*
5) Pathogen + chemical fungicide Beltanol + Biological preparation *T. harzianum*
6) Without pathogen + chemical fungicide Beltanol
7) Without pathogen + Biological preparation *T. harzianum*
8) Without pathogen + chemical fungicide Beltanol + biological preparation *T. harzianum*

The fungus of the biological agent *T. harzianum* was added at a rate of 10 g/ L with the irrigation water in relation to its treatments, as well as the chemical fungicide Beltanol was added in its treatment at a concentration of 0.5% at a rate of 50 mL/ L with the irrigation water until saturation. After completing the experiment, the pots continued to be watered regularly as needed with the addition of the recommended fertilizer. After 30 d, the results were taken to calculate the percentage and severity of infection by measuring the fresh and dry weight of both the root and vegetative parts of plants.

Effect of the efficiency of the biological agent *T. harzianum* and the chemical fungicide Beltanol and their interactions in controlling disease under nursery conditions.

The experiment was carried out in Nurseries Baghdad during the 2021-2022 spring cultivation. The experiment was divided into four furrows, 3 m long, and distance of 1 m between furrows, and each furrow contained 2,000 3-week-old pots, which were randomly distributed to both cultivars Dwarf and dendritic. The experiment was carried out according to

the Randomized Complete Block Design (RCBD) after the 4 treatments were distributed to each furrow and at three replicates for each treatment. The experiment included the following treatments:

1) Beltanol chemical fungicide at a concentration of 50 mL/ L of water
2) Biological preparation *T. harzianum* at a concentration of 10 g/L of water
3) Biological agent *T. harzianum* + Beltanol at a concentration of 50 mL/ L of water
4) The control without any addition.

The pots were watered regularly and as needed with the addition of the recommended fertilizer after completing the experiment. The results were taken after 10 d by measuring the fresh and dry weight of both the root and vegetative parts of plants.

RESULTS AND DISCUSSION

Isolation and diagnosis

The results of sample collection showed indicate that the spread of wilt disease and root rot on *Catharanthus roseus* plants in all the areas covered, from which samples were collected for the growing season 2021-2022 in Baghdad Governorate (Al-Grayat, Al-Sidiyah, Al-Khadra, Al-Ghazaliyah and Al-Dora) and the infection rate ranged between 15-85 % (Table 1). Al-Grayat nurseries recorded the highest infection rate of 85%, followed by Al-Sidiyah nurseries, the percentage of infection was 65%, and then Al-Khadra nurseries had the highest rate of 40%, while Al-Dora and Al-Ghazaliyah recorded the lowest infection rate, which amounted to 15 and 25%, respectively. These percentages of infection cannot be ignored, as they caused great economic losses to the owners of the nurseries and greatly reduced the number of *C. roseus* plants. The spread of the disease also led to the reluctance of some nursery owners to grow this plant (personal contact), The reason for the widespread spread of root rot and wilt disease in the nurseries of the Al-Grayat, Al-Sidiyah and Al-Khadra area may be attributed to the repeated cultivation in the same nurseries and to two spring and autumn growing seasons, which are old nurseries, which led to the accumulation of a number of pathogenic fungi vaccine. As these areas, specialize in the cultivation of *C. roseus* as well as their use of large quantities of organic fertilizers. The sensitivity of the cultivated varieties may have a role in the spread of the disease in the Ghazaliya and Dora regions, perhaps due to the fact that these nurseries are newly established. In addition, it is not excluded that the use of chemical fungicide s has a role in the varying rates of infection among nurseries grown with *C. roseus* plants.

Table (1): The percentage of infection in some nurseries in Baghdad regions**.**

Sample No.	Sample location	Infection rate (%)
1	Al-Grayat	85
2	Al-Sidiya	65
3	Al-Khadra	40
4	Al-Dora	15
5	Al-Ghazaliya	15

The results of isolation and diagnosis of the pathogen from infected plants collected from different nurseries in Baghdad governorate also showed accompanying different types of fungi, the most frequent species in most of the samples were the species belonging to the genus *Fusarium*, *F. solani, F. equiset, F. oxysporum* with percentages of 35, 22.75 and 20.13, respectively (Table 2). The isolation results also showed the presence of some other fungi such

as *Pythium sp, Rhizoctonia solani, Rhizoctonia sp, Aspergillus sp and Penicillium sp* with a frequency of 2.09. These results are in agreement with **(Nejat *et al.*, 2015)**. A number of genera of fungi that cause root and wilt diseases were isolated from a number of ornamental plants, including the genus *Fusarium*. These results are also in agreement with what (**Ayob & Simarani, 2016)** found, where the fungus *F. solani* was isolated regions from the roots of *Catharanthus roseus* plants infected with root rot from the northern of Malaysia. The results of this study are also in agreement with another study, which showed that the fungi *R. solani* and *F. oxysporum* were isolated from a number of nurseries in Karbala and Babylon governorates **(Jubeir, 2002)**. These fungi were diagnosed based on phenotypic and microscopic characteristics. The species belonging to the genus *Fusarium* formed Macroconidia and Microconidia, as well as the length of the conidiophores and its constituent cell, Phialides. Microscopic examination showed that the mycelium is divided. The small conidia of *F. solani* consist of one or two cells oval or spherical in shape, and the larger spores are of crescent shape, just as the fungus *Chlamydospores* have rough-walled spherical shape, **(Lesile & Summerell, 2006)**. Whereas the fungus *F. oxysporum*, it forms colonies of different colors on the PDA medium, starting from white cotton or transparent white, as well as may appear in pink or purple.

The colonies of the fungus *F. equiset* were also distinguished by that the colonies of this type did not give any color or dyes to the agricultural media and were white in color. Conidiophores are short and large conidia divided by 2-5 cells, the apical cell is pointed, elongated and transparent, the foot cell is prominent and distinct, and the chlamydial spores are transparent and spherical produced at the end of the mycelium **(Hami *et al*., 2021)**.

Table (2): The most important types of fungi isolated from the roots of Periwinkle Madagascar plants.

Fungi	Isolate number	Frequencye (%)
Fusarium solani	10	35
Fusarium equiseti	9	22.75
Fusarium oxysporum	7	20.13
other fungi	-	2.09

Test the effect of different concentrations of the chemical fungicide Beltanol on the growth of the biological factor and the pathogen in the laboratory

The results of this test showed that the use of the fungicide Beltanol at concentrations (50, 100, 500, 1000, 1500 and 2000) mg/ L led to the inhibition of the growth of the mycelium of the pathogenic fungus *F. equiseti* by 100% for all the above concentrations respectively compared to the control treatment without addition the fungicide was applied to the PDA culture medium that had 0% inhibition (Figure 1 and Picture 1). This result confirms the high ability of this fungicide to control pathogens, through the nature of its action, as the active substance Chinosol binds with heavy elements and is a complex compound that is difficult to be absorbed by the pathogen, or the fungicide is chelating compounds with copper in the tissues of the host, which facilitates its passage into cells. The pathogen is then released and the pathogen is killed according to what he indicated **(Meister, 2000)**. This result is consistent with what was found by **Radi (2011)**, who obtained an inhibition rate of 100% when using Beltanol at a concentration of (1 and 1.5) mL/L against the pathogenic fungus *F. solani*. This result is consistent with the results of a number of studies and research that indicated the ability of this fungicide to control pathogens, in particular soil pathogens. The treatment with Beltanol at a

concentration of 1-1.5 mL/L led to the inhibition of the growth of the mycelium of the isolates of the fungus *F. solani* and *R. solani* at 100% compared to the control treatment on PDA, **(Radi, 2016)**.

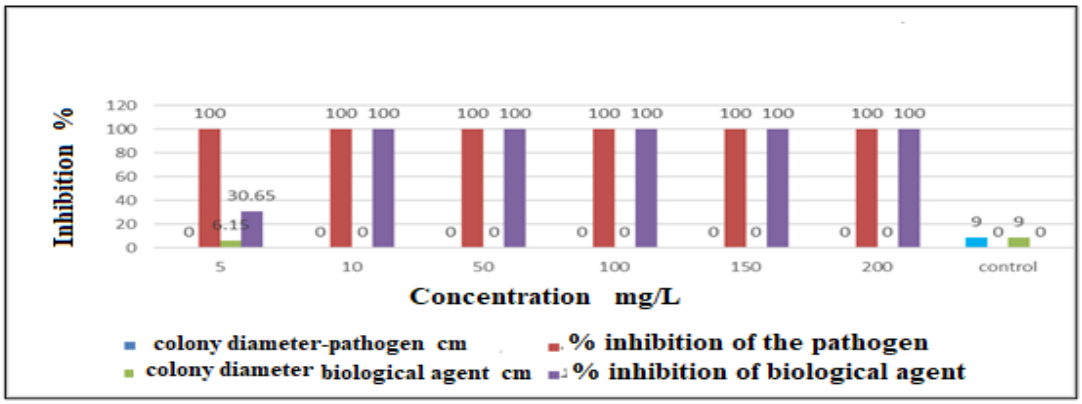
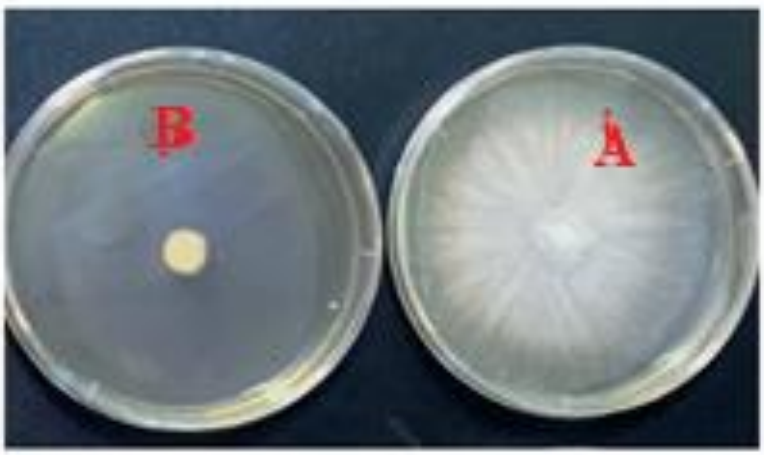


Figure (1): A test of the effect of different concentrations of the chemical fungicide Beltanol on inhibiting the growth of the pathogen *F. equiseti* and *T. harzianum* in the laboratory



Picture (1) The effect of a concentration of 5 mg/L of the chemical fungicide Beltanol in inhibiting the growth of the pathogen *F. equiseti* and *T. harzianum* on PDA culture media.
A- Beltanol fungicide at a concentration of (50) mg/L had no effect on inhibiting the growth of the biological agent *T. harzianum* on PDA culture media.
B- Beltanol fungicide at a concentration of (50) mg/L has an effect on inhibiting the growth of the pathogen *F. equiseti* on PDA culture media.

The results of this test also showed that the use of Beltanol at a concentration of 50 mg /L had a lower effect on the biological fungi and with highly significant differences than the rest of the concentrations used in the test. The percentage of inhibiting the growth of the biological factor *T. harzianum* was 30.65% at a concentration of 50 mg/L and with a colony growth rate of 6.15 cm from the diameter of the dish. Whereas, the concentrations (100, 500, 1000, 1500 and 2000) mg/L gave a percentage of biological factor inhibition that reached 100% and a colony growth rate of 0 cm respectively, compared to the control treatment without adding the fungicide to the PDA culture media, which was the percentage of inhibition 0% and a colony growth rate of 9 cm. We conclude from this that the fungicide Beltanol is one of the least inhibiting fungicide s for the biological fungus *T. harzianum*. Perhaps this is due to the

fact that the fungicide is a systemic fungicide that is characterized by its high specialization against soil pathogenic fungi, especially the pathogens of vascular wilt and root rot diseases **(Al-Adel, 2006)**. This result is consistent with what **Al-Badran (2011)** showed in his study that the fungicide (Carbendazim Swift) is an important fungicide in controlling the disease of palm inflorescence rot. Caused by *Fusarium* sp. and *Mauginiella scaettae Cav*. With an inhibition rate of up to 100%, at the same time, the growth of the colony of the biological fungus *T. harzianum* was inhibited by 82.4%.

Effect of the efficiency of the biological agent *T. harzianum* and the chemical fungicide Beltanol in controlling disease under greenhouse conditions

The results of this experiment showed that all treatments in which the biological agent *T. harzianum* and the chemical fungicide Beltanol were used, either alone or in combination with the pathogenic fungus, reduced the negative effect of the pathogenic fungus *F. equiseti*. It clearly led to a decrease in the rate and severity of infection and a significant increase in the growth parameters of Periwinkle Madagascar seedlings, as it led to an increase in the fresh and dry weight of the vegetative and root parts of Periwinkle Madagascar seedlings of the dwarf variety. Each of these treatments worked with its different mechanisms, whether it was inhibiting, competing, or stimulating the control of the plant or others, which provided protection for the seedlings of Periwinkle Madagascar from infection with pathogenic fungi after 30 d of the pots experiment. As the results presented in Table (3) showed that all the treatments used in this experiment achieved a reduction in the percentage of infection and the severity of infection for the dwarf variety Periwinkle Madagascar plant in different degrees compared to the treatment of the two pathogenic fungi separately.

The treatment of the biological agent when it was interacted with the chemical fungicide Beltanol+*T. harzianum* outperformed the rest of the treatments with highly significant differences in the rate and severity of infection, as it recorded 0% respectively. Followed by the two treatments of the interaction of the biological agent with the chemical fungicide in the presence of the pathogen, as the infection rate and severity reached 10 and 5%, respectively, in the treatment of *T. harzianum*+Beltanol+*F. equiseti*, followed by the treatment of the biological agent *T. harzianum* alone, as the rate of infection reached 20% and its intensity was 10%, followed by the treatment of the chemical fungicide alone, where the infection rate and severity were recorded at 60 and 20%, respectively. This result came close to the control treatment, as there are no significant differences between them, as this treatment recorded the percentage and severity of injury that amounted to 60 and 25%, respectively. This was followed by the treatment of the biological agent with the pathogenic fungus *T. harzianum+F. equiseti,* and the infection rate and severity were 80 and 40%, respectively. This result came close to control by comparing the treatment of the fungicide with the pathogenic fungus Beltanol + *F. equiseti*, which amounted to 90%, but differed significantly in the severity of the infection, reaching 80%, respectively, in comparison with the pathogenic fungus treatment alone, the pathogenic fungus *F. equiseti* recorded the highest infection rate of 100 and 85%, respectively.

Also, the results presented in Table (3) showed that all treatments used in this experiment achieved a significant increase in growth parameters in relation to the fresh and dry weight of the vegetative and root parts of the dwarf variety Periwinkle Madagascar plant compared to the pathogenic fungus treatment. The treatment of the biological agent when used with the chemical fungicide Beltanol+*T. harzianum* outperformed the rest of the treatments with highly significant differences in the average fresh and dry weight of the vegetative and root parts, as it scored 13.50, 3.66, 2.70 and 0.38 g/plant, respectively, followed by the

treatment of the biological agent *T. harzianum* alone, as the fresh and dry weight of the vegetative and root parts reached 12.74, 2.58, 2.68 and 0.36 g/plant, respectively. It was followed by the two treatments of the use of the biological agent with the chemical fungicide in the presence of the two pathogens separately, as the fresh and dry weight of the vegetative and root parts reached 11.86, 3.06, 2.52 and 0.30 g/plant, respectively, in the treatment of *T. harzianum*+Beltanol+*F. equiseti*. This was followed by the treatment of the biological agent with the pathogenic fungus *T. harzianum* + *F. equiseti*. The fresh and dry weight of the vegetative and root parts reached 10.12, 2.2, 1.24 and 0.26 g/plant, respectively. This result came close to treating the chemical fungicide Beltanol alone, as it recorded the fresh and dry weight of the vegetative and root parts at a rate of 8.68, 1.74, 0.95 and 0.20 g/plant, respectively. Followed by the control treatment (without pathogenic fungi), the fresh and dry weight of the shoot and root system reached 7.72, 1.68, 0.78 and 0.15 g/plant respectively, followed by the treatment of the fungicide with the pathogenic fungus Beltanol+*F. equiseti*, where the average reached 7.54, 1.52, 0.72 And 0.14 g/plant, respectively, for the root and shoots in comparison with the treatment of the pathogenic fungus alone, the pathogenic fungus recorded the lowest average of fresh and dry weight of the plant system, but it did not record significant differences with the control treatment in the fresh and dry weight of the root system, as the pathogenic fungus *F. equiseti* gave rates of 6.14, 1.24, 0.68 and 0.12 g/plant. For the fresh and dry weights of the vegetative and root systems, respectively. We conclude from the results of this experiment that the biological agent *T. harzianum* and the chemical fungicide Beltanol, either alone or in combination with the pathogenic fungus, possess the mechanism of supplying the plant with the necessary elements, which facilitated their absorption from the plant after analyzing the organic and inorganic materials surrounding the roots. Which helped to supply the plant with the elements, in addition, the possibility of the fungus *T. harzianum* to produce plant hormones such as IAA **(Yadav *et al.*, 2011)**.

The role of the biological agent *T. harzianum* may come in reducing the incidence and severity of pathogenic fungi because of their inhibitory action resulting from the process of fungal parasitism, competition for nutrients and location, and their production of a number of antibiotics and enzymes that destroy the walls of pathogenic fungi cells. In addition to the secondary metabolites that have a toxic effect on the pathogenic fungus **(Al-Hijazi, 2018)**, the biological fungus *T. harzianum* used its various mechanisms to inhibit the pathogenic fungus and improve the growth parameters of plants. The biological fungi produce a number of antibiotics, including Trichodermin, Trichodermol and Gliotoxin, as these antibiotics reduce the fungal growth of a number of fungal pathogens **(Kuguk & Kivang, 2002)**. The biological fungus also works to produce a number of enzymes such as B-glucans, Chitinase, and Protease, which work on degrading the cell walls of pathogenic fungi, in addition to increasing the plant inducing enzymes such as the enzyme Peroxidase and Polyphenol oxidase related to defense mechanisms in the plant **(Abood & Hakeem, 2016)**. This result is similar to that of **(EL-Mohamedy *et al.* 2015)**. As for the role of the chemical fungicide Beltanol treatment, it worked to discourage the pathogenic fungus, which provided protection for the seedlings of Periwinkle Madagascar from the infection of the pathogenic fungus and led to a reduction in the rate and severity of infection with pathogenic fungi and raising the standards of plant growth by killing spores and fungal cells or preventing them from growing. Thus, it limits the damage it causes to the plant. It was also found that Beltanol works to kill pathogens and fungi in particular, as it is represented by the formation of chelating compounds with copper in the tissues of the host, and this facilitates its passage into the cells of the pathogen to kill the pathogen **(Meister, 2000)**. This result is consistent with the results of a number of researchers

interested in studying the effectiveness of Beltanol, such as the study of the researcher (**Rady, 2011)**.

Table (3): Effect of the efficiency of *T. harzianum* and Beltanol and their interactions in controlling the disease and some growth parameters in the plants of Periwinkle Madagascar of the dwarf variety under the conditions of the greenhouse.

Treatments	Infection rate (%)	Infection intensity (%)	some growth parameters of the dwarf variety under the conditions of the greenhouse			
			Vegetative system		Root system	
			Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Control	0.00	0.00	7.72	1.68	0.78	0.15
F. equiseti	100	85	6.14	1.24	0.68	0.12
Beltanol + *F. equiseti*	90	80	7.54	1.52	0.72	0.14
Beltanol + *T. harzianum*	0	0	13.50	3.66	2.70	0.38
Beltanol	60	20	8.68	1.74	0.95	0.20
T. harzianum* + *F. equiseti	80	40	10.12	2.2	1.24	0.26
T. harzianum	20	10	12.74	2.58	2.68	0.36
T. harzianum* + Beltanol + *F. equiseti	10	5	11.86	3.06	2.52	0.30
LSD	**9.42**	**6.44**	**0.04**	**0.11**	**0.39**	**0.04**

Effect of the efficiency of the biological agent *T. harzianum* and the chemical fungicide Beltanol and their interactions in controlling disease under nursery conditions.

The results of this experiment showed that the treatments used had clearly reduced the negative impact of pathogens and provided good protection for Periwinkle Madagascar plants of the dwarf variety from infection by root rot and wilt disease on Periwinkle Madagascar with significant differences compared to the control treatment (without pathogenic fungi) (Picture 2). The results presented in Table (4) showed that the high effectiveness of the biological agent *T. harzianum* and the chemical fungicide Beltanol in reducing the infection rate and the severity of the pathogenic fungus on the plants of Periwinkle Madagascar of the dwarf variety when used alone or used together without the presence of the pathogenic fungus after 10 in pot experiment. The treatment of the biological agent when added with the chemical fungicide Beltanol+*T. harzianum* outperformed the rest of the treatments with highly significant differences in the rate and severity of infection, it recorded the lowest rate of infection rate and severity of 0% respectively, followed by the treatment of the biological agent *T. harzianum*, which achieved a rate of infection rate and severity of 25 and 10% respectively, followed by the treatment of the chemical fungicide Beltanol, which recorded a rate of 40 and 15% respectively for the percentage and severity of infection, compared with the control treatment, in which the highest infection rate and severity were 60 and 25%, respectively.

The results also showed the superiority of the treatment of the biological agent when it was used with the chemical fungicide Beltanol+*T. harzianum* over the rest of the treatments with highly significant differences in the average dry and fresh weight of the root and the vegetative systems, as it recorded the highest rate of 2.7, 11.7, 15 and 75.3 g/plant,

respectively, for the fresh and dry weight weights of root and vegetative systems, followed by the treatment of the biological agent *T. harzianum*, which achieved an average dry and fresh weight of the root and vegetative systems amounting to 2.1, 9.0, 12 and 70.2 g/plant respectively, followed by the treatment of the chemical fungicide Beltanol, where the average dry and fresh weight of the root and vegetative systems was recorded at 1.2, 5.7, 7.5 and 44.4 g/plant, respectively compared with the control treatment, in which the lowest average dry and fresh weight of the root and vegetative systems was 0.3, 3.6, 6.3 and 41.7 g/plant, respectively.

We conclude from the results of this experiment that the role of the biological agent *T. harzianum* in recording the highest rate of fresh and dry weight of the plants of Periwinkle Madagascar of the dwarf variety may be attributed to the ability of the biological control agent *T. harzianum* to produce a number of antibiotics, including Pyrone, Isonirites and Alkylpyrone, Polyketides, Steroids, Diketopiperazines, Peptibols **(Harman, 2000)**.

As well as antibiotics such as Trichodermol, Trichodermin and Gliotoxin, as these antibiotics reduce the fungal growth of a number of pathogens, including the fungus *Fusarium* **(Radi, 2016)**, or it may be attributed to the inhibitory effect of the biological factor *T. harzianum* through the process of mycoparasitism, competition for nutrients, space, its production of antibiotics (antibiosis) and a number of enzymes, including cellulases, hemicellulases, proteases, B-1, 3-glucans. Peroxidase, polyphenol- oxidase and chitinase against a number of plant pathogenic fungi, including the genus *Fusarium* spp. As well as the secondary metabolites of the biological factor and its active role in inducing the defense system in plants **(Mahdy *et al*., 2011)**.

As for the role of the chemical fungicide Beltanol treatment, as it worked to discourage the pathogenic fungus, which provided protection for the seedlings of Periwinkle Madagascar from infection with the pathogenic fungus and led to raising the growth standards, by killing fungal spores and hyphae or preventing them from growing, thus limiting the damage they cause to plants **(Al-Adel, 2006)**. It was also found that Beltanol works to kill pathogens and fungi in particular, as it is represented by the formation of chelating compounds with copper in the tissues of the host, and this facilitates its passage into the cells of the pathogen to kill the pathogen. This result is consistent with the results of a number of researchers interested in studying the effectiveness of Beltanol, such as the study of (**Al Mayahi, 2020)**.

Table (4): Effect of the efficiency of *T. harzianum* and Beltanol and their interactions in controlling the disease and some growth parameters in the plants of Periwinkle Madagascar of the dwarf variety under nursery conditions.

Treatments	Infection rate (%)	Infection intensity (%)	some growth parameters of the dwarf variety under the conditions of the greenhouse			
			Vegetative system		Root system	
			Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Control	50	25	0.3	3.6	6.3	41.7
Beltanol	40	10	1.2	5.7	7.5	44.4
T. harzianum	30	5	2.1	9.0	12	70.2
T. harzianum+ Beltanol	0.0	0.0	2.7	11.7	15	75.3
LSD	10.5	6.7	0.4	0.4	0.4	0.4

Picture (2): A comparison between the plants of Periwinkle Madagascar of the dwarf variety in the control treatments and plants infected with the cause of root rot and wilt disease in control treatments.

CONCLUSION

The causes of wilt and root rot disease on *Catharanthus roseus* are *Fusarium oxysporum, F. solani, F. equiseti* and *Rhizoctonia solani*, and they appeared in high recurrence rates, the most frequent of which were *F. equiseti*. The chemical fungicide Beltanol possesses a high inhibitory ability against the pathogenic fungus *F. equiseti* on the culture media. The use of the chemical fungicide Beltanol alone or in the form of its combination with the biological agent *T. harzianum* provided protection for *C. roseus* plants from infection with wilt disease and root rot caused by the pathogenic fungus *F. equiseti* and increased plant growth parameters under greenhouse and nursery conditions.

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