

## Record of *Alternaria tenuissima* as a causal pathogen of Leaf Spots in Chard Plant in Basrah, Iraq, and It's *Invitro* Management

Authors:

Alaa O. Manea<sup>1</sup>, Anna D. khamas<sup>1</sup>, Mohammed A. Fayyadh<sup>1</sup>, Hajra Azeem<sup>2</sup>

<sup>1</sup> College of Agriculture, Department Of Plant Protection, University of Basrah, Iraq.

<sup>2</sup> Crop Diseases Research Institute, National Agricultural Research Centre, Islamabad, Pakistan.

Corresponding Author:

Alaa O. Manea

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### **ABSTRACT:**

The chard plant is an important vegetable crop, thus this study aimed to isolate and identify *Alternaria tenuissima* and efficiency evaluation of *Pseudomonas fluorescens*, *Trichoderma viride*, *Trichoderma harzianum*, and *Paecilomyces fumosoroseus* suppression against *A. tenuissima*. *T. harzianum* demonstrated its ability to inhibit the *A.tenuissima* by 63.8%. Also, it reduced the leaf spot number to 0.39 cm/leaf compared to the control that scored 2.19 cm/leaf. The *T. harzianum* increased the leaves and root weight, followed by *P fluorescens* treatment. This is considered the first recorded in Basra, Iraq.

**Keywords:** *Alternaria tenuissima*, *Trichoderma harzianum*, Chard plant, leaf spot disease

### **INTRODUCTION:**

Chard, scientifically referred to as *Beta vulgaris* L. var. *cicla*, goes by the common name of leaf beet in English. Notably, the larger-leaved varieties of this vegetable are recognized as Swiss chard. This versatile vegetable is a vital component of various culinary traditions and offers a essential nutrients that contribute to human well-being. Swiss chard, or simply chard, is celebrated for its nutritional richness. It serves as an excellent source of vital minerals like iron, calcium, magnesium, and sulfur, all of which are crucial for maintaining optimal health. In addition to these essential minerals, chard boasts an array of vitamins, including vitamin C, various B vitamins, and vitamin A. This impressive nutritional profile makes chard a valuable addition to a balanced diet. BVR contains secondary metabolites, called betalains, which are used as natural dyes in food industry and show anticancer activity. (Ninfali and Angelino, 2013) Chard leaves also contain Phytopigments that protects the human body against cancer and improve immune system (Fiedor and Burda,2019). The crop is affected by many agricultural pests, such as insects and diseases like fungal, viral, nematodes, and parasitic flowering plants. However, leaf spot caused by *Alternaria* is a common disease on this crop (source). Chemical pesticides reduce crop infection with many agricultural pests and diseases but affect our

environment. They are hazardous (Khan *et.al*,2018). *A.tenuissima* recored in Malaysia causing leaf spot on eggplant(Nasehi,*et.al.*,2012) In addition, many of them have lost their effectiveness due to development of new strains of pathogens that resist these chemicals fungicide was dangerous on health (Lerox,*et.al.*,2002) As a result of these problems, much attention has been directed toward biological factors (*Pseudomonas fluorescens*, *Bacillus spp.*, and *Trichoderma spp.*) in resistance to Plant diseases are often caused by pathogens, and the soil is a complex ecosystem teeming with diverse microorganisms that interact with one another. These intricate relationships have been harnessed in innovative ways to combat plant pathogens. Among these relationships, mechanisms of antagonism, parasitism, and competition were found between fungi (Prasad *,et.al.*,2013). Bacteria *P.fluorescens* have several mechanisms through which they influence plant pathogens Such as hydrogen cyanide (Voisard *et al.*, 1989) and Siderophore and Pterines, Phenazines, and Pyrroles are considered antibiotics produced by Bacteria (Pyrrolnitrin (Prn) and Pyoluteorin (PLT) such as *P. fluorescens*(Thomashow and Weller,1996). Phenyazin Carboxylic acid (PCA), Diacetylphloroglycinol (DAPG), and Phenyazin Carboxylic acid (PCA) are the primary interface in the field of biological resistance research, and it has recently been used to describe the chlorination

of the genes responsible for the biosynthetic of these compounds (Dwivedi and Johri 2003). At the same time, *Trichoderma* spp. has diverse mechanisms, Such as competition for the site, food, biological antagonism, and enzymatic activity, which affect the plant pathogens (Benhamou, 1993, De meyer,*et.al.*, 1998, Barakat et al., 2007). The study aimed to identify the pathogen cause of leaf spot disease in Swiss chard. And evaluate the efficiency of biological agents in controlling the pathogen in the laboratory.

## **MATERIALS AND METHODS:**

### **1-Isolation and Identification of the Fungus *A. tenuissima*:**

Samples of chard exhibiting symptoms of the fungus were collected from the area between healthy tissue and the brown spots on chard leaves. To ensure cleanliness, the leaves were carefully washed under running tap water to remove any dust particles and subsequently allowed to air dry for a period. Then cut up leaves into pieces of 0.5-1 cm and disinfected with sodium hypochlorite at a concentration of 3% for 1 minute, that it was washed with distilled water to remove traces of the solution and dried on filter paper Whatman NO. 1 was then transplanted into a petri dish with a diameter of 9 cm containing potato dextrose agar PDA. The dishes were incubated at 25°C for seven days, and the growth of the isolates was observed and purified on the morphology base. Characterization of *A. tenuissima* isolated from leaves depending on the external appearance of the colony, such as color, shape, colony diameter, and height using culture media (PDA, PCA), and microscopic characteristics such as size, shape, structure, spores, and other structures according to taxonomic bases. Existing reports in( Ellis, 1971; Woudenberg et al., 2013and Sun,et al., 2023).

### **2-Molecular Analysis:**

The DNA of the isolated fungi was extracted using a gSYNCTM kit, and the primers F: TCCGTAGGTGAACCTGCGG:

R:TCCTCCGCTTATTGATATGC were used to amplify the ITS1-ITS4 gene region. After the amplification process, the electrophoresis technique was used on an agarose gel. The gel was examined with a gel documentation apparatus to determine the success of the DNA amplification process (Tarini .et al,2010). Then, 20 µl of the amplification product for each isolate was sent to the Korean company MacroGen to determine the sequences of nitrogenous bases in the genes used and then match them with the National Center for Biotechnology Information NCBI and record them.

### **3-Fungal Inoculum Development:**

Prepare the inoculum of the biological agents separately, using an appropriate amount of the seeds of local millet (*miliaceum panicum*) were washed to remove dust and dirt. After that, it was soaked for six hours, then the water was removed by placing it on blotter paper. The seeds were distributed in 250 ml glass flasks at a 50 g/ beaker rate. Their nozzles were blocked with cotton plugs. Then the seeds were sterilized with an autoclave. The temperature is 121°C, and the pressure is 15 pounds/in for one hour (Dewan,1989). The flasks containing sterilized millet seeds were inoculated with fungi that were meant to be tested individually, with five discs of 0.5 cm diameter from the fungus on the nutrient medium. The beakers were placed in the incubator at a temperature of 25-+2°C for five weeks, shaking the beaker every 2-3 days To ensure that the inoculum was distributed to all seeds.

### **4- The Antagonistic of Biological Agents Against *A. tenuissima* invitro:**

The antagonistic biological agents were tested against *A. tenuissima*, at the laboratory of Senior higher studies in the Department of Plant Protection / College of Agriculture, following the method of dual culture technique. PDA culture medium was poured into Petri dishes with a diameter of 9 cm and leave for solidification. The plates were divided into two equal parts by drawing a line at the bottom of the plate. Inoculate the center of the first half with a disk of diameter 0.4 cm. Poison was taken from the edge of the colony of the pathogenic fungus half. The *A. tenuissima* second was inoculated with a disc with a diameter of 0.5 cm from the edge of its colony, one of the fungi used in Biological control. Three dishes were used for each treatment, while the comparison treatment was vaccinated Dishes with the pathogenic fungus *A. tenuissima* Alone (Ghisalberti *et.al.*,1990)

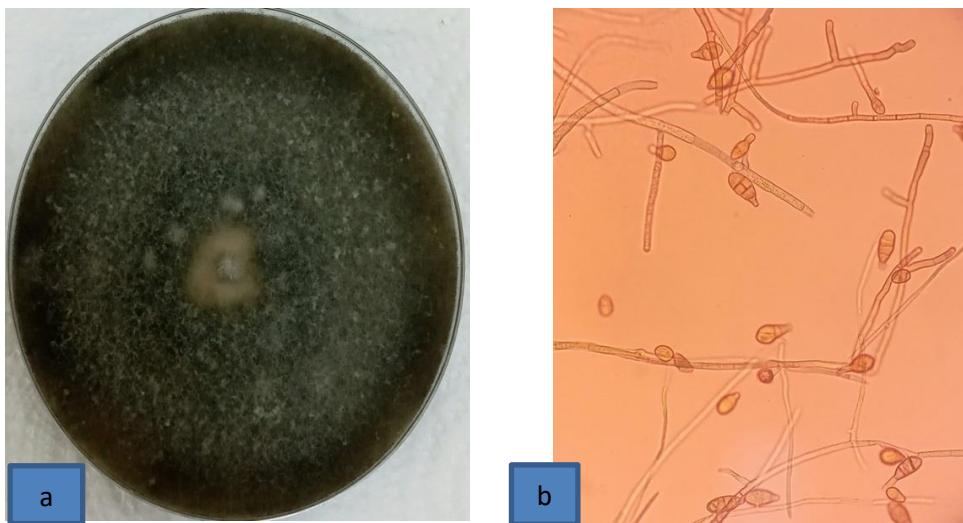
### **5-Pots Experiment:**

In this experiment, a mixture of soil and peatmoss was used in a ratio of 1:3 w/w. The soil was sterilized using a commercial formalin solution by preparing a solution of (1:50 formalin/water). Use the solution in a ratio of 3 liters of formalin solution / m<sup>3</sup> of soil and after ten Days of sterilization were placed in 1 kg plastic containers, in equal quantities, then Distributed into treatments in three replicates (three pots), the soil was inoculated with the agent's biogenic *P.fluorescens*, *T.viride* and *T. harzianum* and the biological agent *Pacillomyces* sp. grown on millet by 1%Weight/weight. The soil was moistened and left for four days after covering it with nylon bags. The chard seeds were sown in each pot, and ten seeds were planted on the date of 10/3/2013. After

the plants grew, they were inoculated with the pathogenic fungus, then the number of spots was

calculated—the fresh and dry weight of both shoots and roots.

## **RESULTS AND DISCUSSION:**



**Figure.1.a. *A.tenuissima* growth in PDA :b. conidia of *A.tenuissima***

### **1. Morphological Identification:**

In Fig.1.a, the growth of *A.tenuissima* in PDA shows the dark green color, and the growth covers the whole Petri dish. Spores were septate with small cylinder shape.

### **2. Screening of Antagonists Against *A. tenuissima* under invitro:**

The antagonistic of biological agents against the pathogenic *A. tenuissima* on PDA. The results of Table (1) indicated the superiority of the biological fungus *T. harzianum* that inhibition pathogen, with a percentage of inhibition of 63.8%, was followed by the bioagent *T. viride*. The percentage reached 55.5%, while bacteria

had a minor effect on inhibiting pathogenic fungi, reaching 33.3 %. This result agreed with what was preached by Al-Saadoun (2011), as we showed that bioagent *T. harzianum* could inhibit the growth of the pathogen *A.tenuissima* in the middle. The inhibition rate reached 73.3%. The anti-fungal ability may be due to its possession.

Several mechanisms affect the growth of pathogenic fungi, such as the production of degrading enzymes on the cell walls of pathogenic fungi, such as chitinase enzymes and B1,3-glucanase, NA Gase, chitinase, acid phosphatase and alginate lyase (Qualhato, *et.al.*, 2013)

**Table. 1: Effect of biological Control Agents in Growth Inhibition *A. tenuissima* Invitro**

Treatments	Inhibition %
<i>T. harzianum</i>	63.8
<i>T. viride</i>	55.5%
<i>P. fumosoroseus</i>	35.3%
<i>P. flourecense</i>	33.3%

### **3. Effect of Biological Control Agents on Chard leaf Spot Caused by *A. tenuissima*:**

The results of the pot experiment are shown in Table (2) in plants inoculated with fungi that the treatment of the biological agent *T. harzianum* led to a reduction in the number of spots. The leaves used in the comparison containing the pathogenic fungus only, which amounted to 2.19 spots/leaf to 0.39 spots/leaf in the treatment of

the biological fungi and the pathogenic fungus, while it was Bacteria have less effect on the pathogen, as the number of spots in it is 62.1 spots/leaf. It may be due to The possession of a biological factor by one or more mechanisms such as competition for food and space, Fungal parasitism, or the production of enzymes and antibiotics, which all work on the Inhibition of pathogenic fungi (Barakat *et al.*, 2007).

**Table.2. Effect of Biological Control Agents in Plant Infestation of Chard Plant with *Alternaria* Leaf Spot**

Treatments	No. of spots(spot/leaf)*
<i>T. harzianum</i> + <i>A. tenuissima</i>	0.39
<i>T. viride</i> + <i>A. tenuissima</i>	0.62
<i>P.flourescens</i> + <i>A. tenuissima</i>	1.62
<i>P. fumosoroseus</i> + <i>A. tenuissima</i>	1.32
Control	2.19

\*all number was mean of 3 replicates

Table (3) shows the best treatments that led to the reduction of pathogenic, thus Increasing the fresh and dry weight of shoots and roots treatment *A.tenuissima* +*T. harzianum*, the fresh weight of the two groups reached 50.1 and 80.0 g/plant, respectively, and the dry weight was 26.0 and 12.0 g/plant, respectively, compared to the control treatment containing pathogenic fungi only for fresh weight. The dry weight of both groups is 80.0, 23.0, 05.0, and 04.0 g/plant.

**Table.3. Effect of biological factors of fresh and dry weight for plants**

treatments	Fresh plant weight*	Fresh root weight	Dry plant weight	Dry root weight
<i>T. harzianum</i> + <i>A. tenuissima</i>	1.50	0.80	0.26	0.12
<i>T. viride</i> + <i>A. tenuissima</i>	1.40	0.38	0.14	0.08
<i>P.flourescens</i> + <i>A. tenuissima</i>	1.69	0.43	0.22	0.13
<i>P.fumosoroseus</i> + <i>A. tenuissima</i>	1.20	0.38	0.23	0.12
Control	0.80	0.23	0.05	0.04
R.L.S.D 0.05	1	0.13	0.15	0.07

As for the ability of the biological factor *T. harzianum* to encourage growth, it may be attributed to the fungus's ability to increase the readiness of some nutrients in the soil, such as phosphorous, potassium, iron, zinc, and copper, and then increase the plant's content of these The elements (Singh and Islam, 2010), or perhaps attributable to the secretion of the biological factor Auxin-like plant growth regulators such as IAA auxins and GA3 gibberellins act in concert with mechanisms Others include increasing the absorption and readiness of nutrients for the plant, as it stimulates the growth regulator GA3 Cell growth and expansion due to increased hydrolyzed starch and other multiply sugars And increasing the softness of the cell walls, and then the expansion of cells in the internodes of some plants, as well as Stimulating IAA production, increasing its formation rate and decreasing its breakdown rate (Hasan,2010).

### **CONCLUSION:**

In this study , *A. tenuissima* was isolated from the symptoms of swisschard leaf spot in Basra , Iraq .Microscopic characteristics was closed to the features mentioned by (woundenburg et al 2013 ,Khan

et.al.,2020). DNA sequence data was applied by using Internal Transcribed spacer (ITS) region of the ribosomal DNA (r DNA), both the sequenced data and the blast (software) revealed 98.71% identity with *A. tenuissima* (Genbank accession No. OP 048982.1). This study gave a clear indication that *A. tenuissima* caused leaf spot diseased on chard based on pathogenicity experiment ,these result are in agreement with another studies that showed *A. tenuissima* causing *Alternaria* Leaf Spot on Sugar Beet (*Beta vulgaris*) in Minnesota, U.S.A.(khan et.al ,2020).And leaf spot in date palm (Manea et.al ,2023).

Pot experiments showed that the treatment of the biological agent *Trichoderma harzianum* led to reduce the number of spot on leaves compare with pathogenic treatment only.Pot experiment also showed that the treatment of biological agent *Trichoderma harzianum* led to reduce the number of spots on leave, and it was the best treatment which increasing of fresh and dry weight of both shoot and vegetative system, the reason for this may be due to Some species of *Trichoderma* have a significant impact on the agricultural environment, making them unique for their use in agriculture. they colonize plant roots without clear negative reactions (Lopez– Bucio et al., 2015) .

*Trichoderma* species have the ability to enhance plant grow by stimulating the plants mechanical defense against pathogens (Harman et al 2004).

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