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FIRST RECORD THE FUNGUS *BIPOLARIS AUSTRALIENSIS* AS A CAUSE OF LEAF BLIGHT DATE PALM IN MISAN AND ATTEMPT TO CONTROL IT IN *IN VITRO* CONDITION

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Abstract

The current study was conducted in date palm groves in Misan Governorate, Iraq, where the fungus *Bipolaris australiensis* was isolated as a cause of palm leaf spot disease. The results of the pathogencity test showed the ability of the fungus to cause the disease, while the use of Swift Sc pesticide (Carbendazim 50%) in the laboratory showed that the pesticide has achieved a high inhibition percentage in concentrations 5, 10 and 15%. Moreover, the concentration of 40% of the alcohol extract for the pomegranate peels also effects in inhibiting the fungus growth compared to other concentrations. Finally, the antagonistic results showed the high ability of *Trichoderma harzianum* to inhibit the growth of pathogenic fungus *in vitro*.

***Keywords*:** Date palm, Misan, spot disease, Carbendazim, inhibit.

Introduction

The *Phoenix dactylifera* belongs to the Arecaceae family, it is of economic importance because its fruits contain sugar, mineral salts, vitamins, and proteins (Kruger 1998). Date palm leaves are infected with several pathogenic fungi, as Abass *et al.* (2007) was able to isolate twenty-two fungal species belonging to eighteen fungus genus from date palm leaves infected with leaf spot disease in the orchards of Shatt Al-Arab in Basra Governorate. Furthermore, Fayadh and Alaa (2008) were able to isolate and identify eight fungal species that cause palm leaf spot disease in Basra, among them the fungus *Bipolaris australiensis* Besides, Alasadi (2010) recorded the presence of the fungus *Bipolaris australiensis* as a cause of palm leaf blight in Basra. However, the presence of pathogenic fungus on adult palm leaves or modern seedlings leads to low growth, less flowering and decreased productivity as a result of affecting the area of the green part of the leaf (Al-kaidy 1994, Djerba 1983). Finally, the current study aimed to investigate one of the causes of palm leaf spot disease in Misan Governorate and the possibility of resistance *in vitro*.

Materials and Methods

- **Symptoms of leaf spot disease on date palm**

The symptoms have been observed and recorded on the date palm leaves at the nursery site of the Misan Governorate Agricultural Directorate, where these trees were considered infected as soon as one or more spots appeared on one or more of its leaves. As well as, the color and extension of the spot on the palm rachis were recorded.

- **The pathogen isolation and identification**

The infected parts represented by the leaves and the midrib of the leaves were cut into small pieces of 0.5 cm length and was washed with running water to get rid of the dirt. Then, it sterilized with sodium hypochlorite solution at a concentration of 10% of the commercial product for 3 minutes, and washed with sterile distilled water and dried with sterile filter paper. All four pieces were transferred to Petri dishes of 9 cm diameter that contain the sterile potato Dextrose Agar media (PDA) with an antibiotic (Chloramphenicol) at a rate of 250 mg / L and drops of lactic acid. The dishes were incubated in an incubator at a temperature of 25 ± 2 °C for 5-7 days, then the fungus was purified on PDA media and identified based on (Ellis, 1976; Domsch *et al.*, 1980) method.

- **Fungus pathogenicity test**

Pathogenicity of pathogenic fungus was tested according to Bachiller and Iiag (1998) method by taking several pieces of date palm rachis from the fourth layer by a length of 15 cm. Subsequently, they washed with running tap water and sterilized with sodium hypochlorite solution 10% of the commercial product for 3 minutes, and then washed with sterile distillate water several times to remove the traces of sterile solution. Moreover, a bore was made for every rachis with a sterile cork borer with a diameter of 0.5 cm, then a disk of an isolated fungus with a diameter of 0.5 cm was placed on the culture media PDA in the bore that was made in the rachis. Each bore was wrapped with a transparent adhesive removed two days after the fungus inoculation. The pieces were placed in sterile glass bottles containing 20 ml sterile distilled water and then the glass bottle nozzle was closed with sterile medical cotton and wrapped with aluminum foil. Furthermore, they were incubated under the temperature of 25 ± 2 °C for a month, where the fungus growth and the development of the disease spot were observed on the rachis every three days. The average radius of the damaged tissue was measured around the injury site and the symptoms were recorded, and when the radius of the artificial injury exceeds 1 mm, it is an indication of the occurrence and development of the fungus infection. The experiment was carried out by taking three replicates, while the comparison treatment was done by placing a 0.5 cm disc in the rachis from the PDA media only.

- **The effect of the chemical pesticide SWIFT SC on fungus growth in the nutrient media**

The PDA nutrient media was prepared and sterile using the Autoclave at 121 °C and 15 lb / in² pressure for 20 minutes. Once the media temperature drops to the pre-solidification, the chemical pesticide SWIFT SC, the active substance Carbendazim 50% was added to the flasks that containing the nutrient media at a concentration of 1, 5, 10 and 15%. Then, the flasks were shaken well for

homogenizing the pesticide with the nutrient media, a 20 ml approximately of those nutrient media was poured for each dish by three replicates for each concentration with comparison treatment (control) containing the nutrient media only. Besides, the dishes were inoculated when the nutrient media was solidification in dishes with a diameter of 0.5 cm from the nutrient media where the pathogen fungus grew in, the dishes were incubated at a temperature of 25 ± 2 °C. Finally, the experiment was stopped after the fungal growth in the comparison treatment reached the edge of the dish where the growth rate was calculated by taking the average of two orthogonal diameters for the growth of fungal colonies that pass through the center of the dish. The percentage of inhibition of fungal growth was calculated according to the following equation:

Percentage of inhibition = average radial growth in comparison- average radial growth in treatment/ average radial growth in comparison x 100

- **The effect of alcoholic extracts of the pomegranate peels on fungus growth in the nutrient media**

Grand *et al.*(1988) method was used in preparing the alcoholic extract, as the extraction process was carried out by crushing dried pomegranate peels in vitro with the solvent (Methanol) at 1 g / 5 ml V / W. The mixture was shaken well for 1-2 hours using the magnetic stirrer, then, the mixture was left in the refrigerator for 24 hours for soaking. It was subsequently filtered through several layers of gauze to remove insoluble particles. The solvent evaporation process was carried out using a rotary vacuum evaporator at a temperature not exceeding 40 °C, this vaporization process continued until the solvent present in the mixture was completely removed, thereby the alcoholic plant extract was obtained in the form of a thick layer, and finally, this extract was dried with the cryodesiccation process. Afterward, the sterile PDA was prepared, and after the media temperature decreased to before solidification, the alcoholic extract was added to the flasks that contained the nutrient media with concentrations of 10, 20, 30 and 40%. the flasks were shaken well to homogenize the extract with the nutrient media, then pouring approximately 20 ml of those nutrient media for each dish by three replicates for each concentration, and with comparison treatment (control) containing the nutrient media only. Also, the dishes were inoculated when the nutrient media was solidification in dishes with a diameter of 0.5 cm from the nutrient media where the pathogenic fungus grew in, the dishes were incubated at a temperature of 25 ± 2 °C. The experiment was stopped after the fungal growth in the comparison treatment reached the edge of the dish where the growth rate was calculated by taking the average of two orthogonal diameters for the growth of fungal colonies that pass through the center of the dish. Furthermore, the percentage of inhibition of fungal growth was calculated according to the following equation:

Percentage of inhibition = average radial growth in comparison - average radial growth in treatment/ average radial growth in comparison x 100

- **Antagonism test between bioagent fungus *T. harzianum* and the pathogenic**

Trichoderma harzianum was used in a double-transplantation method to test the ability of bioagent fungus to antagonistic with the pathogenic fungus. As the petri dish that contained the sterile PDA media was divided into two equal parts, then the center of the first section was inoculated with a 0.5 cm dish of the antagonistic fungus colony *T. harzianum* at four days old. The second section inoculated with a 0.5 cm dish of the pathogenic fungus colony *B. australiensis* with the presence of comparison treatment inoculated in a dish in the center of the dish from *B. australiensis* by three replicates for each treatment and then incubated at 25 ºC. The antagonism was calculated after the growth in the comparison treatment reached the edge of the dish according to Bell *et al.*(1982) scale consisting of five degrees as follows:

1- Antagonistic fungus covers all dishes including the pathogenic.
2- Antagonistic fungus cover two-thirds of the dish.
3- Antagonistic fungus covers half of the dish.
4- Pathogenic fungus covers two-thirds of the dish.
5- Pathogenic fungus covers the entire dish.

The antagonistic fungus is considered effective if the antagonism is between 1-2 degrees.

Results and Discussion

- **Symptoms of leaf spot disease on date palm**

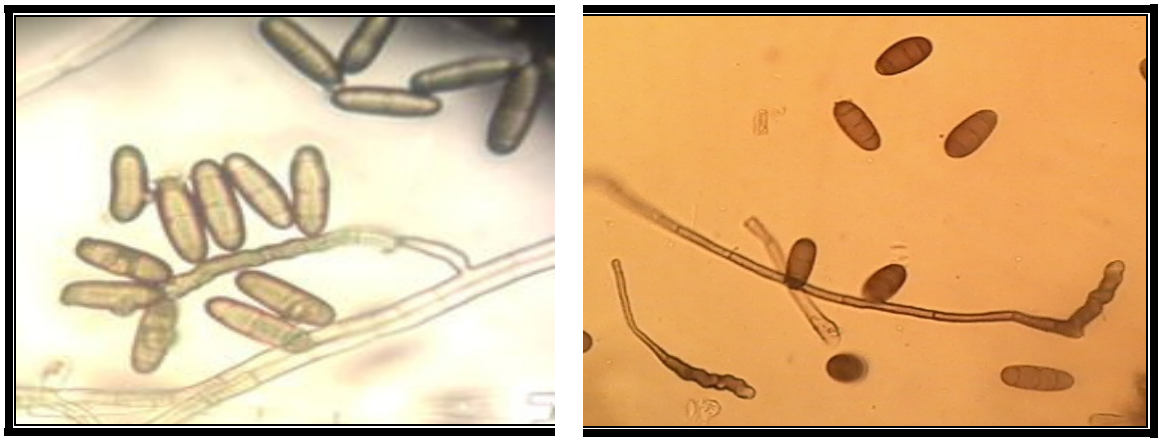
The symptoms that were recorded on the infected palm rachis were in the form of spots or smudges that have a color of brown to dark brown, varies in color depending on the severity of the injury, and may reach black color. The size of the spots ranges between 0.5 to 2 cm, the size of the spots may be greater when two or more spots combine in the case of high severity of the injury.

Picture (1) : Symptoms of leaf spot disease on palm caused by *B. australiensis*

The infection also extends from the rachis towards the leaves forming brown to dark lines as shown in (Picture 1). Additionally, the infection and its severity continue the infection accompanies other fungi such as the *Alternaria alternata* and the *Aspergillus* spp. types. Symptoms can be observed from a certain distance by the leaf losing their dark green color and turning into light color. These results were consistent with Al asadi (2010) study by identified *B. australiensis* as a cause of brown spotting disease on palm leaf rachis in Basra Governorate.

- **Isolation and identification of pathogen *Bipolaris australiensis***

The results of isolation and identification showed that the fungus that causes palm rachis spot disease is the fungus *Bipolaris australiensis*. Through the identification traits of the fungus, as shown in Picture 2, they contain the septate, conidiophore that divided or branched with a reddish-brown color 95-205 mμ and a thickness of 3-7 mμ . Furthermore, the conidiophore arranged and serialized on the conidia, in cylindrical or rectangular shape, pale brown to reddish-brown, divided by a false wall into three cells, the size of conidiophore 7.5^{-10} x 27.5^{-15} mμ .



Picture 2 : Taxonomical characteristics of *B. australiensis* isolated from palm rachis

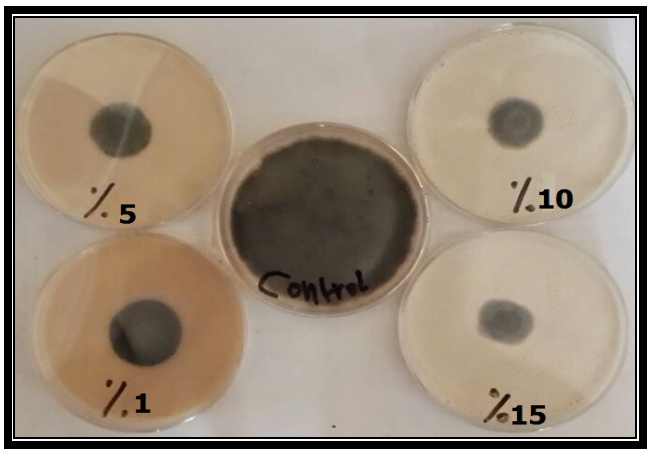
- The pathogenicity test of the causative fungus

The results of the pathogenicity test of the *B. australiensis* (Picture (3)) showed the fungus ability to infect the rachis of the palm leaf, where the radius of the pathological spot extended to a distance of more than 1 mm during a month from pollination and was colored black. However, once making a longitudinal section in the pollinated rachis, it was observed a dark brown and black coloration below the pathological spot. As well as, it was observed that the extension of the coloration to meet with the extension of other pathological spots, forming a black line along with the rachis piece.

Picture 3 : The side of pathological spots, showing the convergence of spots and the formation of a black line

- **The effect of the different concentrations of the chemical pesticide on the growth of the fungus in the PDA media**

The results of the statistical analysis of the experiment in Figure 1 and Picture 4 showed that there were no significant differences between the averages of the inhibition percentage of the fungus growth in the treatment of 5, 10 and 15% concentration which reached 74.44, 75.92 and 82.23%, respectively. Whereas these three averages exceeded with highly significant differences over the inhibition percentage of the fungus growth in the treatment of 1% and over the treatment of control that reached 67.03 and 0.00%, respectively. The use of fungicides is one of the main methods used in controlling the fungus causing plant diseases. Razoki and Samir (2010) were used carboxyl and Raxil in controlling the fungus *Bipolaris* sp. at concentrations 5, 10, 15% on the culture media of potato sucrose agar (PSA).



Picture 4 : The effect of different concentrations of the chemical pesticide on the fungus growth in the PDA media

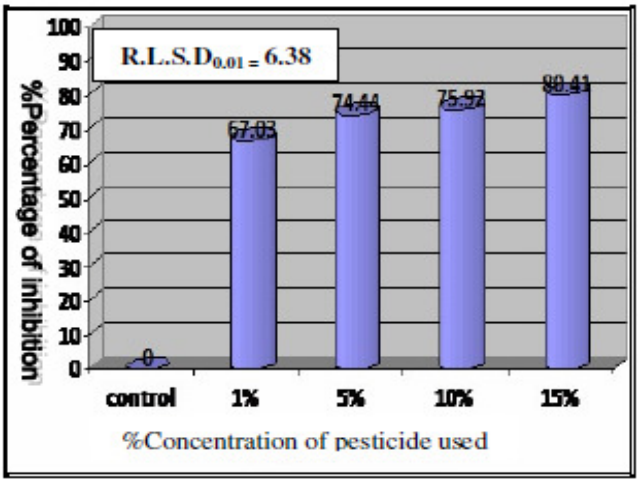


Fig. 1 : The effect of different concentrations of the chemical pesticide on the fungus growth in the PDA media

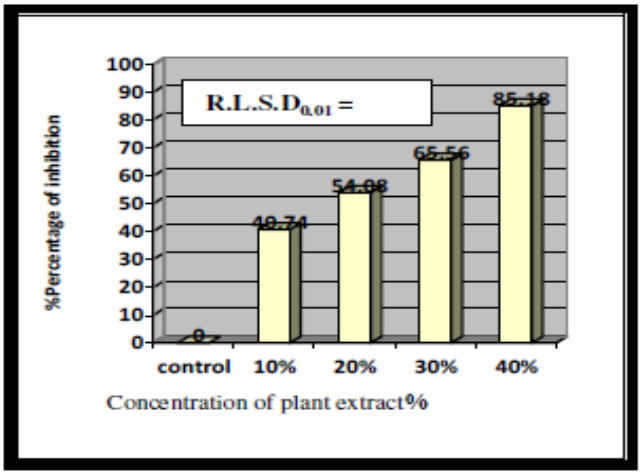
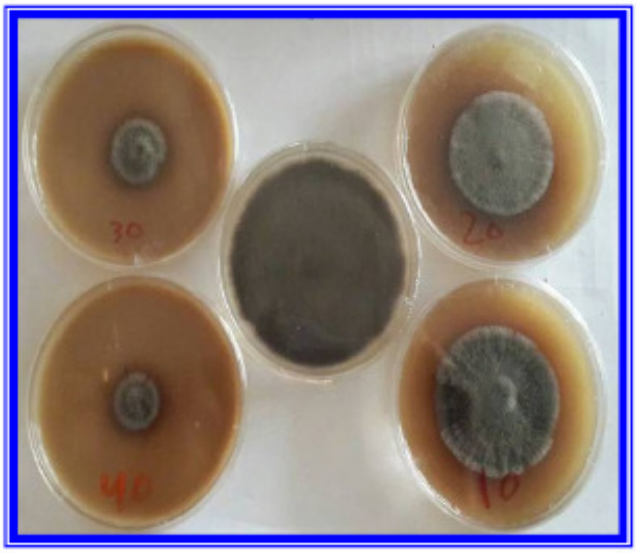


Fig. 2 : Effect of alcoholic concentrations on inhibiting the fungus growth in the PDA media

- **The effect of the different concentrations of alcoholic extracts on the fungus growth in the nutrient media**

The results of the statistical analysis of the experiment in Figure 2, Picture 5 showed that there were significant differences between some averages of the inhibition percentage of the fungus growth on the culture media PDA. The inhibition percentage reached 85.18% in the treatment of 40%, which exceeded over all the remaining treatments, while the inhibition percentage was 65.56% at a concentration of 30%, and the inhibition percentage in 10 and 20% amounted to 40.74 and 54.08, respectively. The use of plant extracts as alternatives to chemical pesticides in the resistance the fungal plant diseases is one of the good solutions to get rid of toxic residues that accumulate in plants because plant extracts are rapidly degrading and without side effects, as the scientific research headed towards experimenting with many plant extracts, including pomegranate peels. The inhibition ability of the pomegranate peels is due to its contains many alkaloids, flavonoids and glycosides that directly affect the cell membranes of fungal cells (Prakash and Indra, 2011).



Picture 5 : Effect of alcoholic extract concentrations on inhibiting the fungus growth in the PDA media

- **Antagonism test between bioagent fungus *T. harzianum* and the pathogenic**

The results of the antagonistic experiment showed that *T. harzianum* has a high antagonistic ability against the pathogenic *B. australiensis* in the culture media PDA, according to Bell *et al.*(1982) scale, as its antagonism reached 1, it is the degree to which the fungus has a biotic, resistant organism as shown in Figure 6

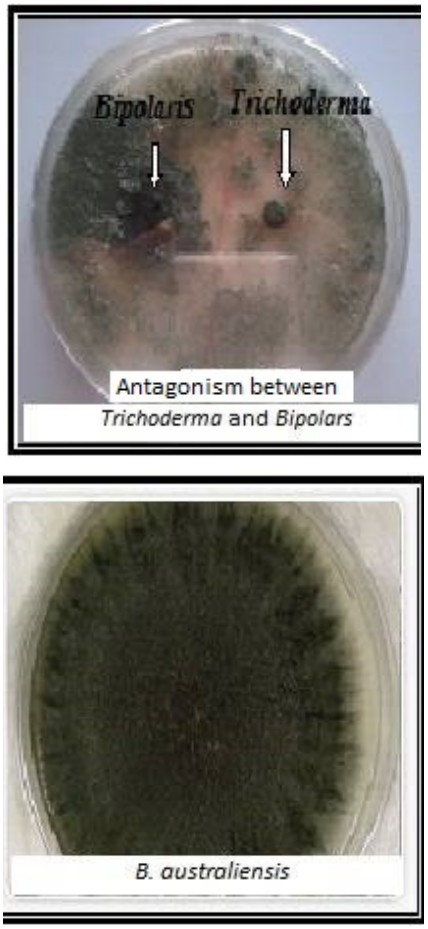


Fig. 6 : Antagonism of bioagent fungus *T. harzianum* against the pathogenic

The reason can be attributed to his possession of the production mechanism of toxic metabolic substances for pathogenic fungus (antibiotics) (Yates *et al.*, 1999), as well as the production of many enzymes that degrading the cell wall for pathogenic fungus such as B-glucanase, Carboxymethyl cellulose, chitinase and glucosidase-α (Sriram *et al.*, 2000; Linglis and Kawchuk, 2002).

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