Diagnosis of causes of cankers on apricot trees Prunus armeniaca L. using PCR technique in some of the Al-Qantara, Al-Baydaa orchards in Karbala- Iraq

Zeinab A. M. Al-Tememe1, Taha M. M. Al-Sweedi1, Kadum mohammed Abdullah1 Chasab K. Jawid2 and Roaa . M Hamzah2

1Department of Plant Protection, Agriculture Collage, University of Kerbala, Kerbala, Iraq
2Department of Horticulture and landscape gardening / College of Agriculture / University of Kerbala/Iraq

Email: Zainab.mohammed@uokerbala.edu.iq

Abstract. The aim of the experiment was to study the causes of the deterioration of the prunus armeniaca L. apricot trees caused by the fungal infection in some orchards of the Al-Qantara, Al-Baydaa / Hosseinieh district/ Karbala province in April 2017 and some methods of control. Results of the field survey showed that Prunus armeniaca L. were decline in percentages ranging from 60-70 degrees. The results of isolation showed the presence of Neoscytalidium novaehollandiae, Alternaria porri on potato dextrose Agar (PDA) medium and identified based on its morphological and molecular characterizations and pathogenicity. This identification is the first record of this fungi affecting in Karbala province of Iraq. The pathogenicity test result showed the ability of fungus to cause decline on the seedlings to Prunus armeniaca L. after two months from inoculation time, the result also showed that N. novaehollandiae was more affected with the pathogenicity which differed significantly from the fungus Al. porri, where the lesion length was 0.66 and 0.56 cm respectively and significant difference from the comparison treatment. These fungi showed toxicity for Prunus armeniaca L. through new branches wilt test, and it was found that the tested fungi caused a significant loss in the amount of chlorophyll a (0.37, 0.44 mg-1 wet weight), These results did not differ from chlorophyll b, which increased by increasing concentration. The results of the Gibberellic acid ( GA3) test with four concentrations (100, 200, 300 and 400 mg L-1) showed an active role in the inhibition process, with superiority of 400 mg. L-1 concentration which recorded a 100% rate of tested fungi. Biological control with T. harizanum and T. atroviride results showed superiority on inhibition fungal growth mycelium and high parasitism ability. The results of the effect of GA3 and Biological control with T. harizanum and T. atroviride had an effect in reducing the lesion length where the superiority of GA3 in 400 mg. L-1 concentration, with the total length of the lesion 0.67 cm by comparison and significantly different from the two types of resistant fungi, which recorded a canker length of less 0.41 cm for T. atroviride and 0.47 cm for T. harizanum with comparison between them and control, which was 1.33 cm long.
1. Introduction

The apricot species of *Prunus armeniaca* L. belongs to the sub-family of Prunoideae, and it is one of the most cultivated species in many parts of Iraq, and comes after the palm in terms of quantity of fruits produced during the months of June and July. Apricots of deciduous trees are of great economic value. Fruits of apricots are fresh, dried or processed, and its fruits are desirable for containing high nutritional value, including carbohydrates, proteins, organic acids, fiber and vitamins, etc. [1]

The average productivity of one tree of apricots 27.5 kg was estimated for the year 2014, a decrease of 3.8% than in 2013, it was 28.6kg, and the highest average productivity was achieved in Baghdad province, estimated at 35.4 kg, while the lowest average productivity was achieved in Basra Governorate in 2014, estimated at 1.6 kg [2]. Apricot trees suffer from many pathogens and are among the most prominent factors specific to the cultivation, especially fungal, leading to the canker of their trunks and thus deterioration of the wood quality produced and deformed as well as the death of branches, stains its leaves and rooting their roots.

The fungus *Neoscytalidium dimidiatum* (Penz) Crous & Slippers attacks the cambium and grows under it causing the canker of stem, the dieback, and the wilting according to the type of tree which leads to the weakening of the cytosolic wood and colored brown, thus cracking the bark longitudinally which is a thinly veiled black powder of spores asexual, it is one of the most important fungus brown rotting of trees and the main cause of canker and the death of branches [3] as result of the mycotoxin secretions that have been diagnosed [4]. Four phytotoxin-induced fungal infections were diagnosed including Glucuronic acid, it is a fungus with a wide family range causing the infection to some species of trees such as *populus nigra*, Willow, Oak and Eucalyptus [5].

The fungus *Alternaria sp.* of mild soft rot fungus, which affects the hardwoods trees [5], was recorded on many trees, including cypresses in Iraq [6], which has a wide family range affecting many trees, including eucalyptus trees [7].

Gibberellic acid (GA3) is one of the most commercially used forms, it plays a role in stimulating stem elongation, seeds germination, breaking the rest phase and helping to inhibit the destruction of chlorophyll, protein and RNA. In addition, Gibberellic acid plays an important role in stimulating the physiological functions of plants and regulating biological processes, including plant growth, photosynthesis, and flower arrangement. [8], as it works on the induction of the defenses of the plant host by stimulating genes responsible for resistance in the plant against the massive numbers of plant pathogens as well as increasing plant growth [9] The biological control of plant pathogens is one of the important methods used to reduce the development of the disease. Fungus *Trichoderma* has been used on many trees, such as citrus and Cypress for both normal and silver types, because of its effective impact in inhibition the growth of fungal spore and reduce the canker of tree stump in the field. Bio-resistance fungus has been known for 70 years by attacking various fungi in its biosphere, and it is one of the most micro-organisms widespread and commonly used in the field of biological control of the world as well as economic importance in the control For a wide range of aetiology, There are currently more than 50 products in the world registered fungi static of fungus *Trichoderma* [10].

Due to the importance of apricot trees which have not been of great importance in the scientific research and limited studies carried out in Karbala governate and in order to minimize the damage that leads to the reduction of economic, commercial and industrial value because of attack by fungi, and due to the importance of apricot trees in Karbala-Iraq and as a result of the deterioration and the absence of a study on the specific canker we decided to do this experiment to identify the cause of the infection and then control.

Several members of bacterium *Bacillus* spp. and fungus *Trichoderma* spp. have been succeeded in combating effectively a wide range of phytopathogens on various agricultural crops in glass houses and fields. This is because of owning diverse control mechanisms such as production range of anti-phytopathogen compounds, nutrient competition, hyperparasitism, and degrading enzymes [14; 15;
16; 17]. Furthermore, there are several other biocontrol factors developed as commercial product such as the Effective Microorganisms (EM1) have been employed to obtain optimal control to soilborne pathogens and to improve plant growth and yields due to consisting of a selection of microorganisms such as Rodopseudomonas spp. that are naturally exist in the environment. These microorganisms produce different substances such as amino acids and carbohydrates that promote plant growth and activate other group of microorganisms that assist in the decomposition of organic matter and increase the fertility of the soil, and produces antibodies against plant pathogens [18]. Thus, as a result of spreading the seed rot and pre-emergence damping-off in most fields of cowpea crop in Kerbala province, this study aimed to isolate and diagnose of the main cause of this disease and assess some local and commercial biocontrol agents in control of the disease.

2. Materials and Methods

2.1 Field survey

A field survey of degraded apricot trees was carried out in four orchards in Al-Qantara, Al-Baydaa district-Husseiniya- Holy Karbala province in April 2017. The infection was identified through cankers on the tree stump in order to know the infection size. Affected trees were classified into five categories depending on the size of damage in randomly selected trees in each orchard, based on the index of deterioration severity according to method [11]. The following relationship was calculated the infection severity in the trees:

\[
\text{Infection intensity} = \frac{T.\text{No. of category (1)} \times \text{Repetition} + \ldots + T.\text{No. of category (5)} \times \text{Repetition}}{\text{T.No. tested} \times \text{Top category index}}
\]

T: tree, No: number

The percentage of infection in the surveyed trees was calculated irrespective of the infection size according to the following relationship:

\[
\text{Percentage of infection} \% = \frac{\text{Infection trees number}}{\text{The total number}} \times 100
\]

The cankers were calculated in stump of apricot trees, two types of canker were identified: canker in prominent spots, and the spots number on the tree was calculated at a height of two meters from the soil surface. While the second type of canker was profound and it has shielding shape, and the dimensions of canker was calculated, in addition the cankers were described in terms of presence or absence of gummous secretions.

2.2 Isolation

Samples of apricot trees P. armeniaca Infected with cankers were brought to the laboratory in order to isolate pathogenic fungi and were isolated according to the following method: The infected parts were washed with running water for one hour, then parts of the areas adjacent to the canker were taken by a sterile scalpel, and parts of dimensions of 5.0 cm from the canker area were taken, and then sterilized using a 1% of sodium hypochlorite for three minutes, then washed with sterile water to remove the surface sterilizer and dried between two sterile filter paper (type of Whatman No.10). The parts were planted in sterile Petri dishes diameter 9 cm contains a sterile culture medium potato extract, dextrose and Potato Dextrose Agar (PDA) with an antibiotic of Chloramphenicol at a rate of 250 mg. L\(^{-1}\) before hardening and 5 parts per dish after their sterilization in the autoclave device at 121 °C and pressure 15 Psi\(^2\) to prevent bacterial growth, dishes were incubated at a temperature of 25-27 °C for 3-5 days. The outgrowth colonies in the
Petri dishes were purified on P.D.A medium, with a 250-mg of Chloramphenicol antibiotic by fungal Hyphal Tip Technique. The isolated fungi were preserved in test tubes containing the sterilized medium of potato extract, Dextrose, Slant agar and sterile until needed to be used in subsequent experiments.

2.3. Polymerase chain reaction (PCR)

The fungal growths around isolates were identified using polymerase chain reaction (PCR), and determination of the nucleotide sequence in the plant viruses laboratory of the Plant Protection Department-College of Agriculture-Karbala University. DNA was extracted (DAN) of pure fungi isolated in 5 days old which grew on the PDA medium of apricot trees as a template in the standard polymerase chain reaction (PCR) for the detection of fungus using Ready TO-GO-PCR Beads Kit provided by Illinois GE Healthcare (USA). The final size of the reaction was 25 microliters, contained the basic components: 1 microliter of ITS1 primers and ITS4 which aims to amplification the area of internal transcribed spacers region (ITS) which located within the small and large unit genes of fungal ribosomes [12].

The products were sent after the amplification process to Macrogen company in South Korea for the purpose of determining the sequence of the nitrogen bases of the studied fungi, results were analyzed using the Chromas program to find out the similarities between isolated and globally recorded fungi, use the Basic Local Alignment Search Tool (BLAST) of (NCBI) the National Center for Biotechnology Information (Gen Bank) and register them with a special accession number.

2.4. Testing the pathogenesis ability of isolated fungi

The experiment was carried out in the greenhouses of Agriculture college - Karbala University, the uninfected seedlings were prepared of apricot Prunus armeniaca L. of 18 months old brought from one of the nurseries in the governorate of Karbala, three branches of each seedling were selected and the wounds and occurred a wound in the bark region by cork drill of a 4 mm diameter in the bark region.

The bark was removed with a sterile needle length of 2 cm and at a height of 5 cm from the soil surface, it has been inoculated with a disc of outgrowth fungi on the PDA culture medium with a diameter of 4 mm. The wound was covered after inoculation with a sterile wet cotton plug.

The disc was then tied by a waxy tape (Parafilm) to avoid contamination of the wound with pathogens [13] each treatment included three replicates.

The results were taken by calculating the average increase in the length of canker after two months of inoculation.

2.5. The toxicological tests of the studied fungi

2.5.1. Test the wilt of young branches

A sterile liquid culture medium was prepared from potatoes and dextrose, then Chloramphenicol antibiotic was added at a rate of 250 mg. L⁻¹, the culture medium was developed in flasks of 500 cm³ of 200 cm² / flask. The culture medium has been inoculated using the discs, it was taken from the edge of the pathogen fungal colonies and incubated at a temperature of 25 ± 2 °C for 14 days. The flasks were placed in a shaker at speed of 40 Pace minutes for 10 minutes. The mediums was filtrated by whatman filter paper, it was placed in the Buchner funnel, installed on the Erlenmeyer flask and then the filtrate was pulled out by vacuum pressure (under complete sterilization conditions). Apricot branches were selected with homogeneous diameters of 15 cm. Each branch had four top leaves and was placed in bottles filled with levels of fungus filtrate (0, 50, 100%). In relation to the comparison treatments, the branches were placed in bottles containing only distilled water. Each treatment included three replicates, and the bottles were placed at laboratory temperature for 48 hours under fluorescent light [14] The results were taken by observing the wilt symptoms on the branches.
2.5.2. Estimation of chlorophyll loss

The loss of chlorophyll a, b, was estimated in the apricot leaves tested by method [15]. Three concentrations (0 %, 50 %, 100 %) were used of the filtrate of the fungus culture obtained as in the paragraph of wilting branches, 100 mg was taken from the weight of fresh leaves in homogeneous shape and size (third and fourth leaf), It was cut into several small pieces by scissors and grind in a ceramic mortar with 6 mL of acetone concentration of 80%, So that the color of the precipitant is free of green color. The filtrate is then separated of the precipitant using Centrifuge at 1600 rpm for 10 minutes and then collected in volume tubes covered with opaque paper to block light of chlorophyll to prevent oxidation and the volume was completed to 6 ml by adding acetone, the optical density (Absorbance) of the precipitant was measured by Spectrophotometer (Type of Shimadzo. UV - 1700) at 645 and 663 nanometers using the following equations below, the concentration of chlorophyll a, b, and the total chlorophyll in the leaves calculated on the basis of mg/ g was estimated as:

\[
\text{Chlorophyll A} = \left[12.7 \ (D663) - 2.69 \ (D645)\right] \times \frac{V}{1000 \times W}
\]

\[
\text{Chlorophyll B} = \left[22.9 \ (D645) - 4.68 \ (D663)\right] \times \frac{V}{1000 \times W}
\]

V: The final size of the precipitant after the separation by a centrifuge
D: Read optical density of chlorophyll extract
W: Fresh weight (gm), the chlorophyll measurement unit is mg/ gram fresh tissue.

2.6. control

2.6.1. Biological control in the laboratory

In the study of the effect of biocontrol, two types of *Trichoderma harzianum* and *Trichoderma atroviride* were used in the resistance of fungus *Ndimidiatum* and *A.pori* in the dual culture technique (DCT). The dishes containing the culture medium (PDA) had been divided into two equal halves by a fixed pen, The center of the first half of the Petri dish was inoculated with a diameter disc of 4 mm, it was taken from the edge of a new colony of the two fungi, and the center of the second half of the Petri dish was inoculated with a diameter disc of 4 mm, it was taken from the edge of a new colony of two types of bio-resistor. Half two dishes were inoculated for comparison with a disc of a pathogenic fungus colony, each treatment included three replicates. The dishes were incubated at 25 ± 2 °C. The degree of parasitism was calculated after three days according to the standard of [16], consisting of five degrees as follows:

Degree 1: Description :Resistant fungus covers the entire Petri dish
Degree2 : Description: Resistant fungus covers of 3/2 of Petri dish
Degree3 : Description: Resistant and pathogenic fungus each covering half the Petri dish
Degree4 : Description: Pathogenic fungus covers of 3/2 of Petri dish
Degree5 : Description: Pathogenic fungus covers the entire Petri dish

Biological resistant which is located within degrees 1 and 2 with high antagonism capacity.

2.6.2 Effect of growth regulator of Gibberelic acid (GA3 ) In the diameter growth of fungus in the laboratory

Gibberelic acid was used to inhibit the growth of fungus after mixing the acid with the sterile PDA medium at concentrations of 0, 100, 200, 300, 400 mg. L⁻¹, mixing well enough to fully melt in the medium before hardening, as well as the addition of 250 mg. L⁻¹ of Chloramphenicol antibiotic and then poured into sterile Petri dishes with 9 cm diameter which had been painted on the base from the outside by the fixed pen two orthogonal diameter, each treatment included three replicates.
Petri dishes were inoculated in its center with a 4 mm diameter disc taken from the edge of new colony growing on the medium of a PDA in five days old of N. *dimidiatum* and A. *porri* by sterile cork drill, the dishes then were incubated at 25 ± 2 ° C. The results were statistically analyzed by calculating the mean of two orthogonal diameter measurements for each growing fungal colony. The following percentage was calculated for the inhibition of fungal spore growth:

\[
\text{% Of the growth inhibition} = \frac{\text{Average of control colony diam.} - \text{Average of treatment colony diam.}}{\text{Average of control colony diameter}} \times 100
\]

2.7. Control in the green house

Apricot seedlings were inoculated as in the test of the pathogenesis ability and then the spore filtrate was prepared for the two types of biological control at a concentration of $6 \times 10^6$ spore.ml$^{-1}$. A barrier was placed during the spraying process for each seedlings to ensure that the spores were not dispersed. Wound region were treated to saturation with a barrier around the seedling during treatment to prevent diffusion the spores to the seedling, while the comparison treatment was left without spraying. The experiment was carried out by three seedlings per treatment according to the length of canker in apricot seedlings after two months of treatment. Apricot seedlings was inoculated as in the test of the pathogenesis ability in a year and a half old. Parafilm was removed after three days of the inoculation, and the wound regions were treated with gibberellic acid at a concentration of 400 mg. L$^{-1}$ until full wetness by three seedlings per treatment, while control treatment was sprayed with water only. The length of canker in apricot seedlings was calculated after two months of treatment. The data were statistically analysed using SAS program using completely randomized design, the mean then was compared with the least significant difference (LSD) at the probability level of 0.05.

3. Results and Discussions

3.1. Field survey

The results of the field survey of apricot trees represented by four orchards in Al-Qantara, Al-Baydaa district-Husseinlia- Holy Karbala province shows that the rates of deterioration in all apricot trees in the surveyed sites were increased by 60-70% and high intensity ranged between 0.70 - 0.93 where there was a presence of canker, It is prevalent in apricot trees as well as having some form of gummosis (Figure 2).

| Region     | %infection | Infection intensity |
|------------|------------|---------------------|
| Orchard 1  | 70         | 0.70                |
| Orchard 2  | 65         | 0.82                |
| Orchard 3  | 60         | 0.92                |
| Orchard 4  | 65         | 0.93                |

The results also show the appearance of two types of cankers: the first cankers is higher spot canker (Figure 1, a), the second type of cankers is the shielding shape (Figure 1, b). Both cankers were found to contain brown sticky gummosis secretions, and the dimensions of cankers of the shielding shape between 33-105 cm tall and 6.5 - 20 cm wide and 6 cm depth.
Figure 1. Percentage and severity of apricot trees infection

3.2. Isolation
The results of isolation from apricot stump showed the appearance of the fungus *Alternaria porri* with a frequency of 60%. The shape of the colony on the medium of the PDA appeared in dark-velvet olivaceous (Figure 2-A). Microscopic testing showed the formation of fungus conidic spores in chains. The shape of the conidial spore is pyriform and microscler, in addition the spore contains longitudinal and transverse barriers (Fig. 2-B).

Figure 2. A- Appearance of *Al. porri* colony on PDA medium
B- spores of fungus

The fungus *N. novaehollandiae* were isolated at a high rate of 82% (Figure 3). The fungus was characterized by its articular spores (Initially transparent and then black with age) as a result of fragmentation of fungal spore, the fungus was formed of pycnidium in the host's bark, mostly melastomataceae. The pycinal stage is called *N. mangiferae*, and the fungus has a arthrosporic (5).

Figure 3. A- Appearance of *N. novaehollandiae* colony on PDA medium
B- articulated phase C- spores of fungus

3.3. Polymerase chain reaction (PCR)
Maximum likelihood phylogenetic tree based on 9 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence showing the relationships of the Iraqi *Neoscytalidium novaehollandiae* with same and other global species sequences available at Gen Bank. The analysis was based on ~ 611 sites and likelihood-ratio tests indicated by Kimura 2-parameter model (Kimura, 1980). Bootstrap values below 50 are not shown. Evolutionary analyses were conducted in MEGA X [17].

3.4. Testing the pathogenesis ability of isolated fungi

![Phylogenetic tree](image_url)

**Figure 4.** Phylogenetic tree constructed using ITS-rDNA sequences, presenting numerous identified *N. dimidiatum* strains stored at Genbank database including that was isolated from *Prunus armeniaca* L.Tree (MF511047; indicated by a black dot).

Maximum likelihood phylogenetic tree based on 8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence showing the relationships of the Iraqi *Alternaria* sp with other global species sequences available at GenBank. The analysis was based on ~ 1188 sites and likelihood-ratio tests indicated by Kimura 2-parameter model (Kimura, 1980). Evolutionary analyses were conducted in MEGA X [17].
Figure 5. Phylogenetic tree constructed using ITS-rDNA sequences, presenting numerous identified *N. dimidiatum* strains stored at Genbank database including that was isolated from *Prunus armeniaca* L.Tree (MF102105; indicated by a black dot).

3.4. Testing the pathogenesis ability of isolated fungi

The test of the pathogenesis ability of fungus isolated from apricots after two months of inoculation, the fungus *N. novaehollandiae* recorded an canker rate of 0.66 cm, which differed significantly of the fungus *Al. porri* which recorded rate of canker length reached 0.56 cm, while they differed significantly of the comparison treatment (control), which gave an average length of canker of zero (Figure 6) and due to losses caused by fungi to trees as previously recorded the fungus *N. dimiditum* and the genus *Alternara* sp. In the economic trees such as pinus, tamarisk, populous, plane trees and other species [5].
3.5. The toxicological tests of the studied fungi
3.5.1. Test the wilt of young branches
Table (2) shows that treatment with isolated fungal filtrate was caused the appearance of symptoms of wilt on the branches of apricot trees have been tested in a different way, it was the more appearance in the filtrate of fungus *N. novaehollandiae* and then in *A. porri*.

The branches began to dry after three days of treatment with fungal filtrate, while no changes were shown on the branches placed in the water. These results are consistent with what he found [14], which showed that the withering was due to the toxicity of the tissues transporter in the fungal filtrate, and thus its reflection on the process of water absorption and its transfer to the branches.

### Table 2. Test of the wilt of new branches with pathogenic fungi filtrates for apricot

| Fungi          | Concentration% | Degree of leaves wilting |
|----------------|----------------|--------------------------|
| *N. novaehollandiae* | 0              | -                        |
|                | 50             | +++                      |
|                | 100            | ++++                     |
| *A. porri*     | 0              | -                        |
|                | 50             | ++                       |
|                | 100            | +++                      |

Based on (14) the wilting of leaves with fungus filtrate was classified into five categories as follows:
- There is no wilting in the branches, + weak wilting, ++ moderate wilting, +++ Clear wilting with leaf color change to gray and brown, ++++ Heavy wilting accompanied by leaf wrapping from top to bottom

3.5.2. Estimation of chlorophyll loss (mg/ g⁻¹ ww)
The results of Table (3) show that the treatment with fungal filtrate caused a reduction of the amount of chlorophyll a and at a rate of (0.37, 0.44 mg. g\(^{-1}\) wet weight) of the fungus \textit{N. novaehollandiae} and \textit{AL. porri} Respectively.

But it differed significantly from the treatment of control which recorded the highest percentage of the amount of chlorophyll a reached (0.69 mg g\(^{-1}\) wet weight). The reduction ratios were increased by increasing the concentration of the fungal filtrate, the concentration gave 100\% of the fungal filtrate the most effective in average of (0.20 mg/ g\(^{-1}\) wet weight), the concentration then 50\%, gave a rate of (0.33 mg/ g\(^{-1}\) wet weight).

With respect to the effect of interaction between fungi and concentrations, the concentration of 100\% and \textit{N. novaehollandiae} fungus registered less amount of chlorophyll a (0.14 mg. g\(^{-1}\) wet weight) compared to the comparison treatment.

Table 3. Effect of concentrations of the studied fungicides filtrates in the amount of chlorophyll a (mg. g\(^{-1}\) fresh weight).

| Concentrate % | 0 | 50 | 100 | Effect of the average fungi |
|---------------|---|----|-----|-----------------------------|
| \textit{N. novaehollandiae} | 0.69 | 0.28 | 0.14 | 0.37 |
| \textit{A. porri} | 0.69 | 0.38 | 0.26 | 0.44 |
| Effect of the average concentration | 0.69 | 0.33 | 0.20 |

Less Significant Difference on the level of probability of 0.05 for the fungus = 0.02, For the concentration = 0.02, for the interaction= 0.03

The results of the table (4) show that there is a significant increase in the loss amount of chlorophyll b with varying degrees of concentration ratios, Which increased with increase it, the concentration of 100\% was registered a ratio reached (0.10 mg. g\(^{-1}\) ww) while concentration of 50% gave a ratio reached (0.15 mg g\(^{-1}\) wet weight) compared to the level of the amount of chlorophyll untreated with fungus filtrate recording an amassed chlorophyll reached (0.35 mg. g\(^{-1}\) wet weight), on the other hand, the tested fungi differed significantly in the loss amount of chlorophyll, and the fungus \textit{N. novaehollandiae} recorded a loss ratio reached (0.22 mg. g\(^{-1}\) wet weight), while the fungus \textit{At. Porri} recorded a loss ratio reached. With regard to the treatment of comparison, it recorded the highest amount of chlorophyll b reached (0.35 mg. g\(^{-1}\) wet weight).Concerning the effect of interaction between the fungus and the concentrations, the concentrations of 100\% and the fungus \textit{N. novaehollandiae} recorded a lowest ratio reached (0.08 mg. g\(^{-1}\) wet weight) compared with the control treatment which registered the highest amount of chlorophyll b.
Table 4. Effect of studied fungi filtrates concentrations on chlorophyll b (mg g\(^{-1}\) fresh weight).

| Concentrate % | 0  | 50 | 100 | Effect of the average fungi |
|---------------|----|----|-----|-----------------------------|
| Fungi         |    |    |     |                             |
| N. novachollandiae | 0 | 50 | 0.08 | 0.18 |
| AL.porri      | 0.35 | 0.13 | 0.12 | 0.22 |
| Effect of the average concentration | 0.35 | 0.18 | 0.10 |

Less significant difference on the level of probability of 0.05 for the fungus = 0.01, For the concentration = 0.01, for the interaction= 0.02

The results of Table (3&4) show The total reduction in chlorophyll a and chlorophyll b increased with the concentration of these leachates as an inevitable result to contain the tested fungi farms on chlorophyll-containing toxic compounds by inhibiting them As a result of the accumulation of some of the intermediate compounds such as Protoporphyrin for the formation of chlorophyll, as well as activation of Chlorophyll enzyme by the fungal and broken toxins of green plastids, Production percentages of chlorophyll [9].

36. Control of the pathogen
3.6.1. Biological control in the laboratory
Both T. harizanum and T. atroviride achieved high antagonistic ability against studied fungi at laboratory conditions (Fig. 7). The two species did not differ significantly from each other, antagonistic ability reached 1.9 on the Bell scale, this may be due to the biotic resistor produces enzymes that decomposition the cellular wall of pathogenic fungi, such as Protease, Esterase, Phosphamidase, and other enzymes [18], or because of the secretion of inhibitory substances against pathogenic fungi, thus limitation it [19], as well as the clump and adhesion of the biotic resistor spores on the fungal hypha of pathogenic fungi and thus decomposition [9].
Figure 7. Antibiotic ability of two types bio-resistors on the two pathogenic fungi
AL : Al. porri • N : N. novachollandiae • Th : T. harizanum • Ta : T. atroviride

3.6.2. Effect of growth regulator of Gibberellic acid (GA3) In the diameter growth of fungus in the laboratory

The effect of four concentrations of Gibberellic acid (100, 200, 300, 400) mg. L\(^{-1}\) was tested, as well as the treatment of the comparison on the growth of fungal spore of pathogenic fungus. Table (5) shows that all concentrations have an active role in the inhibition process. The most influential was the concentration of 400 mg. L\(^{-1}\) registered a rate of 100%, the fungi among them did not differ significantly in the inhibition of growth rates.

The average effect of the interaction of Gibberellic acid concentrations and fungus showed that the fungus did not differ significantly with each other at the concentration of 400 mg. L\(^{-1}\) registered the highest inhibition rate of 100% for the tested fungus. It has been found that algebraic acid plays an important role as a stimulant or catalyst of the plant defense mechanisms against fungal pathogens [9], as well as its ability to induce resistance to fungi, including Aspergillus flavus [18].

Table 5. Effect of GA3 acid concentrations (mg.L\(^{-1}\)) on diametrical growth of N. novachollandiae and A. porri

| Concentrate mg.L\(^{-1}\) Of GA3 | 0   | 100 | 200 | 300 | 400 | Effect of the average fungi |
|-------------------------------|-----|-----|-----|-----|-----|-----------------------------|
| Fungi                         |     |     |     |     |     |                            |
| N. novachollandiae            | 0.00| 51.45 | 75.87 | 85.82 | 100 | 62.62                      |
| A. porri                      | 0.00| 59.41 | 77.64 | 81.64 | 100 | 63.73                      |
| Effect of the average concentration | 0.00| 55.43 | 76.75 | 83.73 | 100 |                            |

Less significant difference on the level of probability of 0.05 for the fungus = 1.39, For the concentration = 2.20, for the interaction= 3.11

3.7. Control in the green house

In the study of the effect of gibberellic acid and the two types of biotic resistor of T. harizanum and T. atroviride on apricot seedlings in the length of canker after two months of inoculation with the
tested fungi, shown in the table (6) which showed a reduction of the length of canker after treated with gibberellic acid at the concentration of 400 mg L\(^{-1}\) and biotic resistors. Gibberellic acid showed a significant increase in the length of the canker, which was 0.67 cm by measurement and significantly different of \(T.\ atroviride\) which registered a lower rate measuring the length of canker of 0.41 cm, while the \(T.\ harizanum\) bio-resistor registered a rate of 0.47 cm compared to the treatment of control, which gave the length of canker of 1.23 cm.

Concerning the fungus, the fungus \(N.\ novaehollandiae\) was exceeded significantly on the fungus \(A L.\ porri\) reached (0.74, 0.65) cm respectively, while the treatment of control registered the highest of canker length.

With regard to the effect of the interaction between the treatments and fungal pathogens was found that all the treatments had a significant effect in limitation of the canker length, the treatment with the Gibberellic acid and the fungal pathogens showed the highest effect with a significant difference between them compared to the treatment of the control and also treated with the resistance biotic resistors. The length of ulceration of the seedlings treated with the biotic resistor \(T.\ atroviride\) and fungus \(N.\ novaehollandiae\) registered 0.41 cm and registered with the fungus \(A L.\ porri\) registered a ratio reached 0.42 cm.

On the other hand, the biotic resistor \(T.\ harizanum\) and the fungus \(N.\ novaehollandiae\) gave the canker length reached 0.51 cm while gave with the fungus \(A L.\ porri\) 0.44 cm, these results coincided with the wide range of the effect of gibberellic acid on a large number of fungi causing the canker symptoms in the shoot system and act as catalysts for the mechanisms of plant protection against pathogens and reducing fungal infections [19].

Table 6. Effect of fungicide GA3 and biological control on length of the canker after two months of inoculation in plastic house

| Fungi              | 0          | GA3 400 | T.a | T.h | Effect of the average fungi |
|--------------------|------------|---------|-----|-----|----------------------------|
| \(N.\ novaehollandiae\) | 1.33       | 0.72    | 0.41| 0.51| 0.74                       |
| \(A L.\ porri\)      | 1.12       | 0.63    | 0.42| 0.44| 0.65                       |
| Effect of the average treatment | 1.23       | 0.67    | 0.41| 0.47|                            |

Less significant difference on the level of probability of 0.05 for the fungus = 0.02, For the concentration = 0.02, for the interaction= 0.04

It is known that the biotic resistor \(Trichoderma\) is an important economic importance in the field of control of a large and wide range of pathogens due to a number of enzymes, it produces a small amount of enzyme \(Exochitinas\) to its biosphere, which works to decomposition the cellular wall and breaking the walls of fungus, thus producing different materials, including Oligomers, where it works to stimulate the production of enzymes, including starting the fungus \(Trichoderma\) parasite on the host [19], as well as that the fungus \(Trichoerma\) has a highly competitive on the nutrients a result of its rapid growth and reproduction at high speed, and it has a good inoculation power being able to the control on the wounds induced oxidation of the cellular lipids and proteins [9].
4. Conclusions
The present evaluation clearly indicated the role of gibberolic acid and native biological control agents (T. harzianum, T. atroviride) in the reduction of canker which it caused by N. novaehollandiae A. porri, fungus, therefore can be used as alternative means to manage the diseases caused.

5. Acknowledgements
Authors would like to express their department of plant protection/University of Kerbala/Agriculture College for providing the facilities utilized in accomplishing of this research project.

References
[1] Nahal I 2003 Tree science directorate of books and university publications (Aleppo, Syria: University Aleppo).
[2] Al-Nuaimi H A et al 2008 Evaluation of the efficacy of water and alcohol extracts of E. callidulensis in inhibiting the growth of positive Gram positive bacteria from patients with pharyngitis and tonsils. Iraqi J. Sci. 49 82-89.
[3] Johnson S W et al 2002 Managing Sooty canker (Nevada, USA: University of Nevada).
[4] Al-Khairu A. N. 2009 Diagnosis of some fungus Nattrassia mangiferae and Phoma exigua (Mosul, Iraq: PhD thesis. University of Mosul)
[5] Altememe Z A et al 2019 Occurrence, identification, pathogenicity and control of Neoscytalidium dimidiatum fungus, the causal agent of sooty canker on Eucalyptus camaldulensis in Kerbala province/Iraq Plant Arch. 19 (1) 31-38.
[6] AlBayati J M 1988 Diagnosis of the causes of the blight on Cypress trees Cupressus sempervirens in the areas of Nineveh and Hammam al-Aalil and some methods of resistance (Mosul, Iraq: MSc. Thesis Mosul University).
[7] Crous P W et al 1989 Newly recorded foliage fungi of Eucalyptus spp. South Africa Phytopathol. 21 85-88
[8] Schalau J 2002 Plant immune Systems (Arizona, USA: Cooperative Extension Yavapai)
[9] Al-Dabagh M N 2012 A study about Neoscytalidium dimidiatum and Neoscytalidium dimidiatum on Eucalyptus in Nineveh (Mosul, Iraq: MSc. Thesis Mosul University).
[10] Frelav D R 2005 Commercialization and implementation of biocontrol Ann. Rev. Phytopathol. 43 337-359
[11] Large E C 1969 Measuring plant disease Ann. Rev. Phytopathol. 4 9 – 28
[12] White T J et al 1990 PCR Protocols a guide to methods and applications (San Diego, USA: Academic Press).
[13] Filer T H 1968 Sycamore canker caused by B.theobromae Phytopathology 59 76-78.
[14] Mohammed N Y 1999 The importance and resistance of some fungi associated with degraded eucalyptus trees in the Nineveh forest (Mosul, Iraq: PhD thesis University of Mosul).
[15] Bell D K et al 1982 In vitro antagonism of Trichoderma. species against six fungal pathogen Phytopathology 72 3-9
[16] Kumar S et al 2018 MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol. 35(6) 1547-1549.
[17] Aboud, H A. 2008 Induction of cowpea seedling Vigna unguiculata using Indoleacetic Acid. Gibberelic acid in the resistance of fungus Aspergillus flavus (Kufa, Iraq: MSc. Thesis University of Kufa).
[18] Harman S H et al 1996 Differential Expression of Trichoderma harzianum chitinase during mycoparasitism Phytopathology 86 981-985.
[19] Abed S H et al 2013 Biological antagonistic between the fungus Rhizoctonia solani and Trichoderma spp causes black scurf disease in potato crops in the laboratory. Yemeni J. Agri. Vete. Sci. 1(1) 64-75.