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Investigation inhibitory effect of the fungus Alternaria filtrate on the growth of the types of bacteria used under study

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***Abstract*---**The present investigative study revealed the inhibitory effect of Alternaria isolates on selectively tested gram-positive and negative bacteria used in this study. Isolate-4 was distinguished by its ability to induce the highest inhibitory effect among the four isolates on E. coli bacteria, followed by isolation-2 as the inhibitory diameter of two isolates was (14 and 10.5) respectively, while the inhibitory diameter of the two isolates (4 and 2) was less. Effect on Proteus Vulgaris bacteria by reaching the inhibitory diameter of 12 mm to isolate-4, followed by isolate-2, isolate-3 and finally isolation-1, as it reached respectively (11.5, 9 and 7) mm, while no significant effect was observed for the four isolates on Bacillus substiles except for the two isolates (3 and 1), they had a clear effect, as it reached a diameter Inhibition (7 and 9), while no effect on staphylococcus aureus was observed.

***Keywords*---**alternaria filtrate, e.coli, proteus vulgaris.

Introduction

Alternaria is a common natural pollutant in the decomposing remains of fruits and vegetables, as it is one of the most common organisms left over after harvesting and has widespread in the soil, food store dust, and decomposition products of organic compounds. It also intrudes on different plants causing losses depending on the severity of the infection and the first description of the disease is leaf spot *Alternaria* (Alalem. 2018). The nature of the parasitism of the different

types of *Alternaria* varies, some of which are not specialized towards a single plant host, a Brassica, which affects different families of flowering plants, jungles, oil plants, several oil-producing plants, and a wide range of plants of the cruciferous family, while others have a specific specialization towards a single plant host such as Stria, the isolation that affects the mandarin trees does not affect the rough lemon plant, and vice versa, as for the fungus *Alternaria*.

It was found that the species that affect the beans and soybeans cannot infect other plants within the legume family or others, and the Alternaria fungi infect the vegetative parts of the remainder, as the mushroom are spread during the growing season by the wind (Jawetz., 2014) The fungi Ternaria Ternata and Ascochyta faba were the most isolated fungi from the leaves of the pea plant for the period from February to May, when studying some areas in Nineveh governorate. Infection may be transmitted from fruits to seeds, as the fungus Alternaria is considered one of the fungi associated with the seeds (storage fungi), as it retains its vitality and susceptibility to infection for up to 15 months from storage under laboratory conditions and may preserve its vitality after 24 months of storage as it was isolated from the seeds of the remaining species at a rate of It is higher than other fungi, which makes it a source of primary infection, as well as contaminated soil, and the seeds show symptoms of infection through the presence of spots on the surface of the seed, which distinguishes them from apparently healthy seeds, as the fungi accompanying the seeds affect the viability of the seed and the percentage of germination in addition to its role in causing the primary infection (Forbes et al., 2012).

The *Alternaria* mushrooms produce a large number of toxins, which are chemical compounds that are affected by the physical and chemical factors surrounding them, and that the heat and pressure reduce the concentrations of toxins produced by the fungus *Alternaria* in the powder of the sunflower, as each of the species produced by the fungus *Alternaria* has an optimum temperature for its production in the required quantities and at lower or higher temperatures than the number of toxins produced decreases, and light also affects the formation or production of mycotoxins (Vukovic., 2017). In a study conducted on seven isolates, alternariol (AOH), and alternariol monomethyl ether (AME) from the fungus of *Alternaria* were shown according to their ability to produce toxins (Kostenko., 2017). When measuring the number of toxins in micrograms/grams, dry weight, that exposing them to continuous light leads to a reduction in the levels of its production of fats, while Adding some chemical compounds to the agricultural environment inhibited the growth of fungi and the vital structure of tenuazonic acid (TeA)(Cooper., 2014).

Material and Method

Preparation of the toxic filtrate: The four *Alternaria* fungus isolates were grown on a Dox shook medium with zinc sulfate [5mg/liter] were added. The sterile medium was supplemented with the antibiotic streptomycin [40mg/L in 500 ml conical flasks at 125 ml per inoculum flask with a 0.6 cm diameter disc from the edge of a culture surface]. The fungi grew until day 5 of age and the flasks were incubated at 25 ± 2 C for 21 days in Potato Dextrose Agar (PDA) medium. On day-5 the medium was filtered with a morselin cloth and centrifuged using a HIGH

SPEED18 MSE type coolant centrifuge (Hassan., 2011). At a speed of 10,000 rpm for 10 minutes and at a temperature of 4°C to facilitate the flow of the toxic filtrate through the bacterial filter with a diameter of 0.45µm for sterilization. Test the filtrate free of bacteria by making a smear on a petri dish containing the agricultural medium (Bashir., 2017).

Extraction and purification of mycotoxin: Zinc sulfate-additive Czapek Medium (CZD) used for isolating 4 of the *Alternaria* alternata fungus at concentration [5 mg/L]. Sterile medium supported with the antibiotic streptomycin [40 mg/L] was placed in a 500 ml conical flask of 125 mL. PDA inoculum flask of 0.6 cm diameter tablets taken from the edge of the developing fungus colony on the medium (Kaura., 2017). At the age of five days, the flasks were placed in an incubator at a temperature of 25 ± 2 C for 21 days, then the fungal culture was filtered using a piece of cloth and run by a chilled centrifuge using a centrifuge refrigerated at a speed of 10,000 rpm for ten minutes at a temperature of 4 C and then concentrated Edwards Modulyo filtrate using a type lyophilizer Another quantity of the filtrate was collected and it was concentrated at a ratio of 1:10. The original concentration and a certain amount of concentrated filtrates were sterilized and used to test the effect of Bacillus subtilis, Staphylococcus aureus on two types of bacteria positive for the Gram stain (WGO, 2011). Proteus Vulgaris, Escherichia coli, and two Gram-negative bacteria.

Purification of mycotoxin

A 2.8 Using hydrochloric acid PH the filtrate was adjusted to pH Then the filtrate was placed in a separating funnel and was extracted three times using ethyl acetate, and the water present with the acetate extract was discarded using anhydrous sodium sulfate. Degree (2.8) Then it was extracted three times using benzene followed by the acidity of the filtrate to pH The extraction was also three times using ethyl acetate (Sakamoto, 2012). The water associated with benzene and ethyl acetate was disposed of in the final stage using anhydrous sodium sulfate crystals (Nagaveni., 2010). The three samples were collected and dried at laboratory temperature and then dissolved with acetone to separate them (Mushtaq., 2018).

Effect of filtrates of fungi isolates on the growth of bacteria

The effect of filtrates of the four fungi isolates on bacterial; where tested on the growth of two Gram-positive bacteria *(Bacillus subtilis and Staphylococcus aureus)*, and two Gram-negative bacteria (*Proteus Vulgaris and Escherichia coli)*. It was obtained from the Department of Life Sciences / College of Science as it was grown inside tubes containing the nutritious broth nutrient agar for a period of (18-20) hours and then planted on Petri dishes container with nutrient agar incubated for two hours and then treated with disks of sterile filter paper Whatman No.1 A diameter of 0.5 cm was dried after treating it with the four sterile isolates. As for the comparison treatment, the treatment tablets were used with sterile distilled water (Sakamoto, 2012). The dishes were incubated at a temperature of 37 m for 24 hours according to the diameter of the inhibition area by measuring the diameter of the inhibition area minus the diameter of the paper used (Nagaveni., 2010).

Detection of pectin enzymes in the culture media of the fungus *Alternaria* (Pectic enzyme clicosidis pectic lyase)

The reduction aggregate-editing method was used as a method to determine the reduced aggregates liberated from the activity of the enzymes of pectic hydrolase (polyketurenoens) and pectic-lipase (polygalacturonase-transaminase). The production of the enzyme in the culture medium: Use Dox hook medium to grow Ternia fungus isolates (1-2-3) with the substitution of sucrose with another carbon source (apple pectin). The sterile medium (with streptomycin, 100 mg / L) in 250 ml conical flasks at 100 ml per bottle of vaccination, each disc beaker of mushroom farm 0.6 cm from rim Fungal PDA heat grown on a medium 25±2 for 15 days the medium was filtered with a piece cloth, and it was centrifuged (4000 rpm for 20 minutes at 4°C) in a refrigerated.[15]

Method

Prepare the reaction mixture from 1 ml of a base material subject to the action of enzymes (polyacetate buffer, sodium pectate) at a concentration of (1%), and add 1ml of a buffer solution with hydrogen number (8) Tric-Hcl Buffer with a pH of (5) and, And (1) ml of the prepared crude enzyme and incubated the reaction mixture for 3 hours at a temperature of 30 ° C. Add (1) ml of the reagent solution (Benedict's solution) to (1) ml of the reaction mixture in a test tube and put the tube in a water bath at boiling point for a while. For 10 minutes, after cooling, mix the solution well and dilute it to 10 ml with distilled water [16]. The spectrometer was used to determine the absorbed light intensity of the solutions at the reference length of 600 nm.

Result and Discussion

The results showed that there is an ability of the isolates of the fungus *Alternaria* to inhibit some types of gram-negative and positive bacteria. It was also observed that there is a variation among the effects of these filtrates. *E. coli* recorded the highest inhibitory diameter with isolate-4; as the diameter of the inhibition was 14mm, followed by filtrate-2 which is equal to 10.5mm, while the isolate filtrate-1 had the same inhibitory effect on bacteria *E. coli* which reached 9 mm, then the effect of fungus leachate on bacteria Proteus *Vulgaris* was observed Gram-positive bacteria, except for bacteria *Bacillus subtitles*. The effect of isolation (1 and 3) was a clear inhibitory effect on it, as the diameter of the inhibitor reached 7 and 9, while no effect on *Staphylococcus aureus* was observed. Then, the effect of the fungus leachate on Proteus bacteria was observed. As we note the uniqueness of isolation 4 in its ability to inhibit this type of bacteria as well, as it reached the diameter of inhibition 12.5 then isolation No. 2 amounted to 11.5 mm, followed by isolation No. (3) 9 mm and finally isolation No. (1), as the diameter of the inhibitor reached 7 mm, while we note that there is no clear effect of the four isolates of the fungus *Alternaria* on Bacillus subtilis positive bacteria for the stain of Gram, except for bacteria The effect of isolation (1 and 3) was a clear inhibitory effect on it, as the diameter of the inhibitor reached 7 and 9, while no effect of Staphylococcus aureus was observed for the two isolates (4, 2) on bacteria.

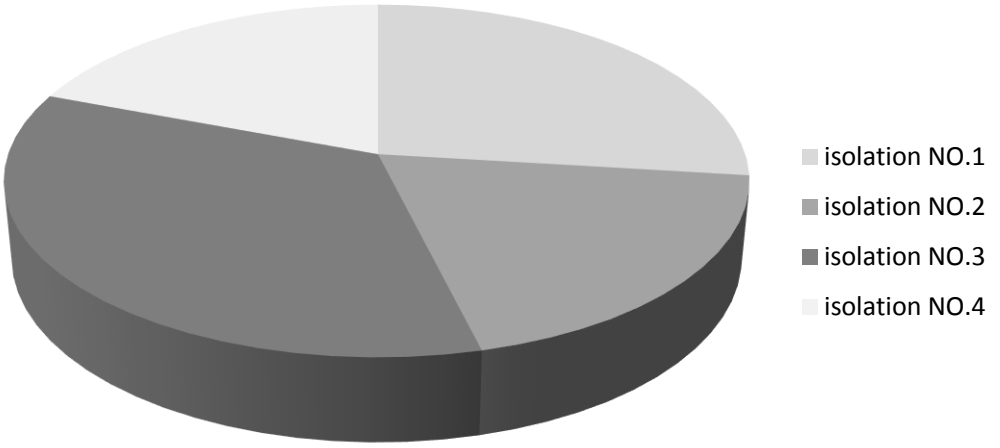

Figure 1. Effect of *Alternaria* isolate extract on the growth of *E. coli* bacteria

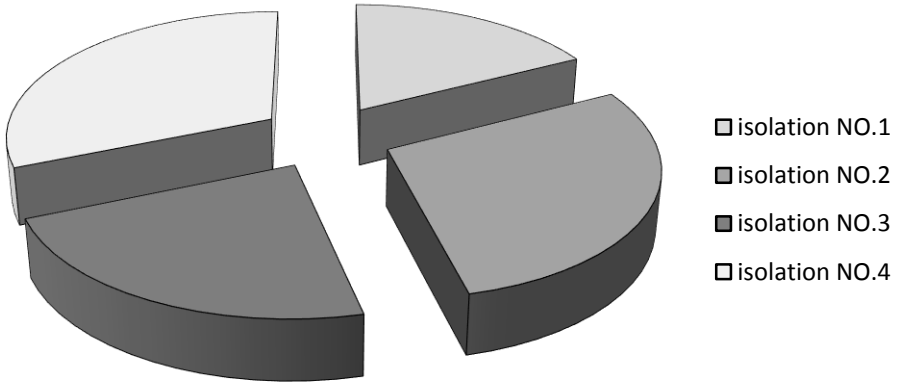

Figure 2. Effect of *Alternaria* isolates extracts on the growth of *Proteus spp.* bacteria

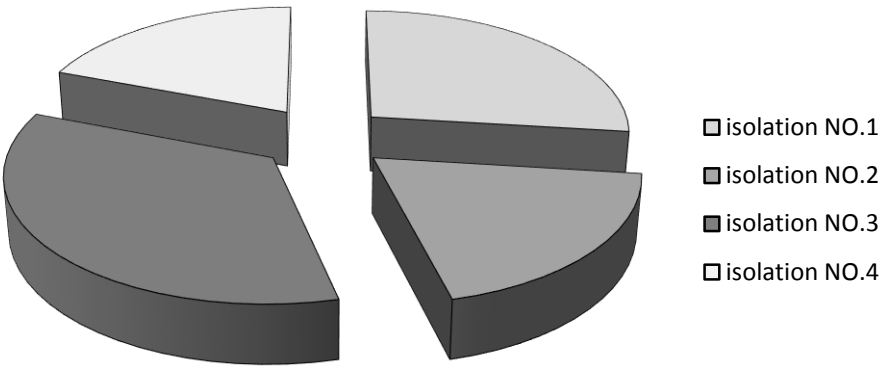

Figure 3. Effect of *Alternaria* isolate extract on the growth of *Staphylococcus Aureus* bacteria

Detection of pectin enzymes in the culture medium of the fungus

The presence of the two enzymes polygalacturonase and polygalacturonase transaminase were found in the three fungi isolates-1, -3, and -4; and in different proportions, where the isolate-4 was the most common enzyme-producing isolates, which is the polylacturinase, as its absorption intensity reached (0.49) which significantly ($p<0.05$) differ from isolates-1 and -3, where the intensity of absorption for them was 0.37 and 0.42, respectively. While isolate-3 was the most productive isolate for the polyclonal enzyme - trans aliments, as its absorption intensity reached (0.45) compared to the two isolates-1 and -4, which reached a level of 0.35 and 0.40, respectively (Bose., 2009).

Detection of pectin enzymes in the culture medium of the fungus *Alternaria* and the ability of the fungus to produce bacterial enzymes that degrade cell walls as well as its secondary role in infection as it works to change the color of the plant tissue to brown, which causes the appearance of infection symptoms according to from this research we conclude Separation of toxins produced by the fungus *Alternaria* cell and observation of their effect on laboratory animals. The need to pay attention to studying wild yeasts due to their utmost importance in their ability to produce deadly toxins, and from there to study the extent of their effectiveness as antibiotics in facing the damages arising from pathogenic yeasts and some types of bacteria and fungi. This difference in the effect of the filtrates of the four fungi isolates on the growth of negative and positive bacteria species for the Gram stain used under study is due to the presence of protein substances or protein glycoproteins produced by this thought by the secondary metabolism process that may have a direct effect on the growth of microorganisms and this agrees with What he found Herrero (Herrero., 2015).

Conclusion

Naturally isolated fungi *Alternaria* filtrate has been shown an inhibitory effect on common bacterial pathogens. These effects have been attributed to the presence of different compounds in the filtrate. These results could be a template for the isolation of new products with antibiotic activity from natural remedies.

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