Sys Rev Pharm 2020; 11(4): 27 – 31
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Isolation and Identification Fungi Caused Rot of *Capsicum annum* Fruit and Study Ability to Produce Protease and Lipase

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Article History: *Submitted: 20.01.2020* *Revised: 09.03.2020* *Accepted: 03.04.2020*

ABSTRACT

The study aimed to isolate the types of fungus that cause the rotting of green pepper fruits , 30 specimens of green pepper (Capsicum annuum L.) were collected from the local markets of Mosul. Seven fungal strains of these fruits were isolated: Alternaria alternate (78) isolation and Aspergillus Niger (29) isolation and Aspergillus flavus (14) Geotrichum candidum (11) Isolation and Rhizopus stolonifera (10) Isolation and Rhizoctonia solani (6) Isolation and Penicillium sp. (4) Isolation, with different percentages The highest percentage of isolation was for fungi Alternaria alternate 51.3%.
to investigate some of the plant's disease factors, the ability of isolated species to produce the proteolytic enzyme was investigated by isolated fungi, Alternate Alternaria was found to be the most common fungus of the enzyme, followed by Aspergillus Niger but Geotrichum candidum cannot produce this enzyme .

As to produce lipolytic enzyme it has been observed that the fungus Penicillium sp. ,Rhizopus stolonifera, Alternaria alternate and Aspergillus Niger had the ability to produce it, while Rhizoctonia solani, Aspergillus flavus and Geotrichum candidum did not show the ability to produce it.

Keywords: the fruits of green pepper, Fungi, Protease, Lipase.

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DOI: 10.31838/srp.2020.4.06

INTRODUCTION

The green pepper plant belongs to the Solanaceae family, which includes tomatoes, potatoes and other crops. It is grown in various types of soils. Green pepper is an important vegetable because it contains large amounts of vitamins, especially vitamins A and C. And multiple phenol, chlorophyll and carotenoids, as well as many essential oils. Green pepper is used fresh or cooked, and is used with vegetables to prepare soups, and dried pepper is used as a spice for cooking purposes and in medicine it is a strong and repelling stimulant and maintains health. In addition, it prevents heart disease and prevents blood clots and has anti-oxidant properties.

The term Spoilage refers to the reproduction of unwanted microorganisms in food and rendering them unfit for human consumption. And microbial activity is important in food spoilage. There are a large number of organisms that cause the formation of pepper fruits. The most important fungi are Penicillium, Aspergillus, Alternaria and some zygotic fungi such as Mucor and Rhizopus. These types of fungi produce a number of blackboards that spread anywhere in the areas of fruit growth and transportation, so any damage may occur. Fruits lead to infection in the stages of harvest to consumption. It has been shown that fungi enter the host tissue through openings such as stomata and lentils and through the healthy epithelial layer through the formation of growth tubes. Some fruits may bear many nurses, which may cause infection and multiply during storage, thereby reducing the value of the marketing product.

Aspergillus niger, Fusarium moniliforme, Fusarium oxysporum, Colletotrichum aeianum, Bipolaris Zeicola, and Aspergillus niger were isolated in the highest proportions. Pests and diseases are important factors affecting optimal production due to massive loss in both areas in the field and in storage. Diseases in the soil include And those that affect the roots and stems, and others that affect the leaves and fruits, and these diseases are caused by types of bacteria, fungi, and viruses, which differ from one country to another depending on climatic conditions and the virulence factors of pathogens. Rot is defined as food spoilage, which reduces its value or may become useless. This term is caused by microorganisms that penetrate food and make it unsafe for human consumption. There are many organisms that cause rot infest pepper and fungi are the most important, as well as the characteristics of mold fungi. Where there are many fungi Penicillium, Aspergillus and Alternaria, and some Zygote fungi, especially Mucor and Rhizopus, produce many blackboards and are present on many fruits on the farm that benefit from the presence of any damage or bruises, so attack this fruit at any stage from the field until it reaches the consumer.

Pepper fruit infections have been recorded by fungi Alternaria alternata, Aspergillus niger, Rhizopus stolonifer, Fusarium solani, Geotrichum candidum,. Mucor has also been recorded by other studies of fungi infections with fungi Aspergillus niger, Aspergillus nidulans, Aspergillus fumigatus, Penicillium sp., Oxysporium. Fusarium.

Microorganisms observed on the surface of fruits and vegetables that may be sourced from the soil produce many exogenous enzymes dependent on them for their diseases, including pro tease and slipcase enzymes, which play an important role in the mechanics of their virility during the growth cycle of the pathogen, as secreted proteins facilitate the ability of the fungus to penetrate and spread In the host and analyze the mechanical barriers of the host.

In addition to its role in colonizing the host by suppressing the defense mechanisms of the host, some protein-analyzing enzymes are qualitative control systems that are responsible for disrupting host defenses and facilitating bacterial infections. There are many protein-dependent enzymes that have regulatory roles for specific responses to pathogens to environmental changes and encourage the occurrence of types Bacteria, as these enzymes are important components of the protein quality control system, which is responsible for maintaining cellular defenses, which are disrupted, thereby facilitating bacterial infections, and successful infection

reduces Rah and the presence of mechanisms in plant pathogens that lead to tissue penetration and colonization.

1. Sample Collection

30 samples of green pepper fruits were collected from different local markets of Mosul.

2. Isolation of fungi from the fruits of the green pepper plant

Fungi were isolated from the fruits of the green pepper plant after washing them with water by transferring 1 cm 2 from the area affected by the fungi and sterilized with a solution of Hypo Sodium Chlorate 1% and then washed with sterile drip water and dried between two sterile filtration papers, and by 5 pieces and planted on Petra dishes container on the food medium Potato Dextrose Agar containing Antibiotic streptomycin sulfate at 50 mg / L and incubated for 7 days at 28 ° C.

3. Diagnosis of isolated fungi

After the growth of fungi on the food medium, small portions were taken by means of a needle from the edge of each fungal colony and individually planted on food media for the purpose of obtaining and diagnosing pure fungi farms, and after the growth of these fungi were examined by an electron microscope and diagnosed depending on the diagnostic keys for the fungi.

The percentage of fungi frequency was calculated according to the following formula:

$$\text{Frequency percentage} = \frac{\text{The number of fungal species colonies}}{\text{The total number of colonies of fungal species}} * 100$$

4. Protease production test by isolated fungi

Prepare the medium from the following materials:

Nutrient agar nourishing agar 950 ml and gelatin as a base material. Prepare the gelatin solution with a concentration of 8% in distilled water and sterilize separately, then add to the sterile nourishing nests by 5 ml / 100 ml and distribute in Petra dishes and leave to freeze, transfer a tablet From each fungus by punching the cork with a diameter of 0.5 mm and placing the center of the petri dish containing the prepared medium and by 3 plates for each fungus incubating the dishes at a temperature of 28 ° C for a period of 7 days with continuous viewing

Protease was detected by adding the Fraziers reagent, consisting of: Hgcl25 g, HCl 20 ml, distilled water 100 ml. Add the reagent to the plates, leave for 5 minutes, then pour, then investigate the transparent aura around the fungal colony producing the enzyme. The activity of the fungus producing the enzyme was measured by measuring the width of the aura measured in millimeters

5. Lipase enzyme production test by isolated fungi

Bring the medium used to test Lipase production from the following components: Peptone 10 g, 0.1 CaCl2.2H2O g, NaCl 5 g, Agar 20 g, Tween 20 10 ml as the base material, Distal Water 1000 ml, sterility of the conductor device, sterilized Tween 20 separately and cooled and then added to the rest of the sterile media components by 1 ml / 100 ml, pour into Petri dishes and leave to freeze, then transfer a disk from each fungus by a cork perforate with a diameter of 0.5 mm and place the center of a Petri dish containing the prepared medium and by 3 plates for each fungus incubated the dishes at a temperature of 28 ° C for a period of 7 days with continuous viewing.

The ability of the fungus to produce the lipase enzyme was investigated by the appearance of a white precipitate or white crystals under the colony(9).

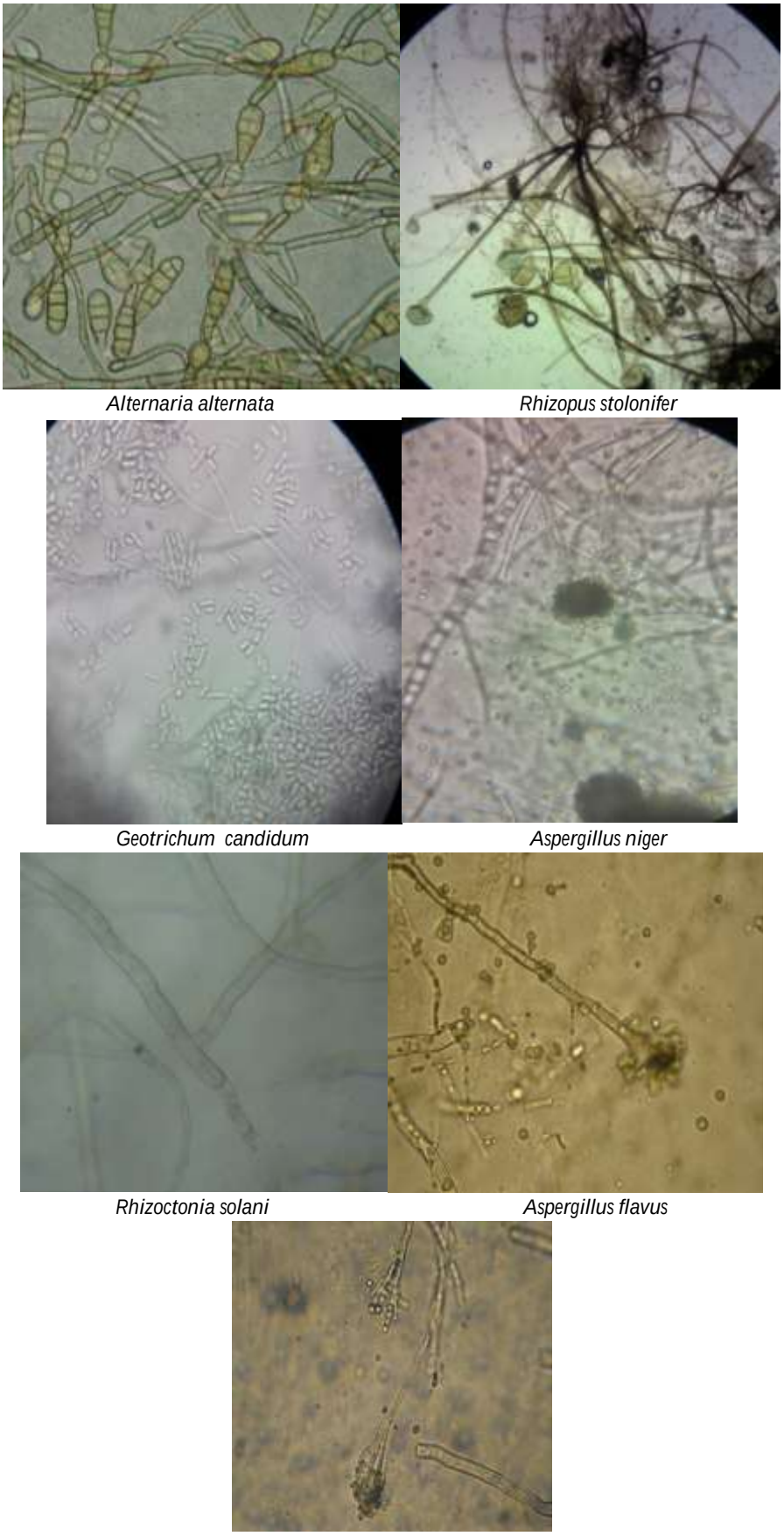
RESULTS AND DISCUSSION

Seven fungi genera were isolated from the green pepper fruits taken from the local markets of Mosul, Alternaria alternata, Aspergillus niger, Aspergillus flavus, Geotrichum candidum, Rhizopus stolonifer, Rhizoctonia solani and Penicillium sp. The highest percentage of isolation for the fungus Alternaria alternata was 51.3%, followed by the fungus niger Aspergillus 18.1% and the fungus Aspergillus flavus 9.2%, respectively, as shown in (Table 1).

Table 1: Shows the percentage of isolation of fungi

The percentage of isolation	fungi
51,3	*Alternaria alternata*
18,1	*Aspergillus niger*
9,2	*Aspergillus flavus*
7,2	*Geotrichum candidum*
6,6	*Rhizopus stolonifer*
4,0	*Rhizoctonia solani*
2,6	*Penicillium* sp.

The frequent occurrence of these fungi, especially the alternata Alternaria fungus, on pepper fruits indicates that it possesses several virulence factors that enable it to invade the fruits and cause injury events that appear in the form of black spots. The various fungi that lead to damage to the fruits and the reproduction of the fungus in their tissues, including the toxins of the lesser secreted from the fungus Aspergillus flavors. Say excreted substances may affect these other types of fungi which reduces the appearance on the proportions of the fruits of pepper or may be due to the production of pepper plant virulence factors are resistant to these types of fungi compared to other species.



Alternaria alternata *Rhizopus stolonifer*

Geotrichum candidum *Aspergillus niger*

Rhizoctonia solani *Aspergillus flavus*

Penicillium sp.

Figure 1: Shows the fungi isolated from the fruits of green pepper

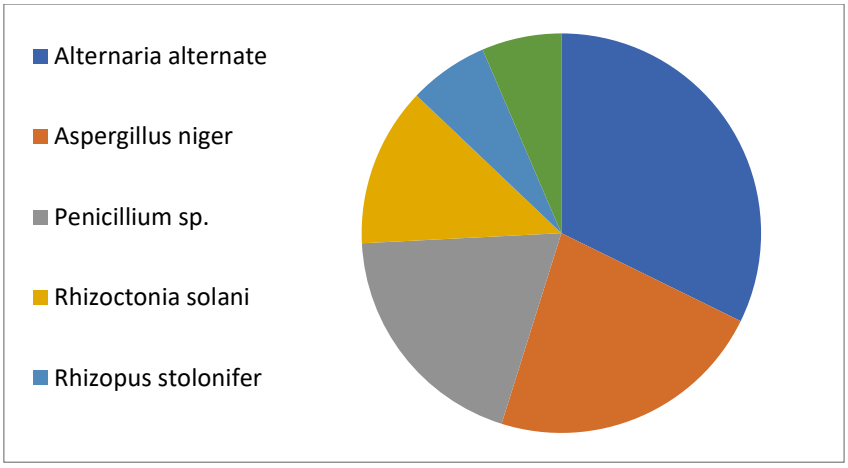


Figure 2: Shows the Protease enzyme production by some fungi.

The results indicated that the alternata Alternata fungus on the medium used resulted in a transparent aura around the colony with a diameter of 5 mm, where the most common fungi were produced by the enzyme followed by the fungus A.niger and a diameter of 3.5 mm then the fungus sp. Penicillum with a diameter of 3 mm and fungi solani Rhizictonia, Rhizopus and A.flavus and diameters of 2 mm, 1 mm and 1 mm, respectively, while the fungus Geotrichum candidum was not analyzed for the protease and may be a sweep of the fungus Alt. Atternata The fruits of green peppers in abundance compared to the rest of the fungi are due to the production of protease enzyme in a greater amount than the rest.

Table. 2: the susceptibility of fungi to lipolysis

production of lipase	Fungi
+	Alternaria alternata
+	Aspergillus niger
+	Penicillium sp.
+	Rhizoctonia solani
-	Rhizopus stolonifer
-	Aspergillus flavus
-	Geotrichum candidum

production of lipase+
Not to produce fungi for the lipase enzyme-

The second table shows the susceptibility of fungi to lipolysis, where it was observed that some fungi led to the analysis of fats such as fungi sp. Penicillium, Rhizopus stolonifer, Alt.alternata, and A.niger. The rest of the fungi, such as solani Rhizoctonia, A.flavus, and Geotricum candidum, did not appear to be susceptible to lipid analysis. The reason may be that these fungi may produce the Lipase enzyme in very small quantities or not excrete them permanently, either the fungi that have been produced The enzyme is considered one of the factors of its ferocity, disease, and injury to fruits, as it was a protease-producing enzyme, as well as its Lipase enzyme, which is able to analyze fats, as fats are components of cell walls as well. Thus, whenever the fungi are able to produce enzymes that analyze cell walls, their disease increases because they are considered the first line for invading the fungus. For fruiting and sweetening Its cell walls and thus enable access to internal tissues and absorption of nutrients, including analysis and corruption, and thus become unfit for the consumer and cause economic losses due to this fungus, which may be sourced from the field and after harvest during transport and storage especially for imported types (3).

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