زانكۆی سەلاحەدین - هەولێر

Salahaddin University_Erbil

College of Agricultural Engineering Sciences

Morphological Identification of fungi associated with potato tubers

Research project

Submitted to the department of (plant protection) part of the requirements for the degree of B.A or BSc in (plant protection sciences)

By:

Muhammad Nareman Omar

Supervised by:

Dr. Ashna Othman Muhammad

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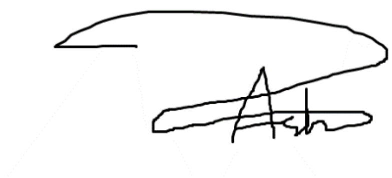
CERTIFICATE

This research project has been written under my supervision and has been submitted for the award of the BSc. degree in Agricultural Science – Plant Protection with my approval as a supervisor



Signature Name: Dr.Ashna othman mohammad
Date: 30/3/2024

DEDICATION

I dedicate this review article to:
The sake of Allah, my creator, and my master, my great teacher and messenger: Mohammed (may Allah bless and grant him.), who taught us the purpose of life.
I dedicate all my efforts and struggles of my educational life to my dear parents; without them I’m meaningless.
Also, I devote the work of this internship report to respectable and honorable teachers

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Summery

Potato, one of the most important vegetable crops, is feeding the growing global population. It has an enormous economic outlet, however; fungal pathogens associated with seed tuber cause huge losses in its production. In this study total of 8 genera of fungi have been identified, that are (*Alternaria solani* , *Phytophthora* sp , *Fusarium* sp ,*Colletotrichum* sp, *Clonostachys* sp ,*Rhizopus stolonifer* ,*Rhizoctonia solani* and *Fusarium solani*) were included. The highest isolation frequency was attributed to *Fusarium* sp by 26.56% whereas the lowest isolation frequency was displayed to *Rhizoctonia solani* by 1.56%.of the total isolates.

Accordingly, the result of the pathogenicity test of pathogenic fungi on the It had Significant differences among the isolated fungi the biggest rot volume *Fusarium solani* was rot volume (360.71) mm volume rot symptom of *Colletotrichum sp* and *Rhizopus stolonifer* .(54.82,45.79)mm respectively.

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1. Introduction

1The potato (*Solanum tuberosum* L.), a species of the Solanaceae family, is valued as one of the most significant vegetable crops (AL-Taey et al., 2019). Many studies have been carried out to improve the worldwide cultivation of potatoes, with both quality and quantity (Aydin et al., 2016).

3Numerous diseases, such as bacteria, fungi, viruses, nematodes, and phytoplasmas, may damage potato plants and result in large losses. (Kumar et al., 2017). The potato crop is susceptible to numerous diseases that can destroy it before and after harvest. In the field, during transport, storage, and commercialization, fungal pathogens result in economic losses. (Eken et al., 2000). Potato late blight, given on by *Phytophthora infestans*, an Oomycota member (Govers et al., 1997). causes significant yield losses in most worldwide potato-growing regions. Typical leaf symptoms include concentric rings around dark brown to black lesions (Van der Waals et al., 2001).

Alternaria solani early blight causes large yield losses in most potato-growing regions of the world(Van der waals et al., 2001). Dry rot, which is caused by isolated, dark, irregular, sunken lesions on the surface, develops in infected tubers (Pscheidt, 1985). The first sign of Rhizoctonia disease is black scurf, which appears later in the growing season and is identified by the development of irregular, black sclerotia on the tuber that vary in size. It is possible for the lesion area size and number of infected tubers to change, and in cases of severe infections, tubers may become cracked and deformed. (Frank and Leach, 1980). The fungus *Helminthosporium solani* causes silver scurf, a detrimental disease to potatoes that has a significant economic impact (cullen et al., 2001).

Each of the fungi's identification and the verification of their pathogenicity on potato tubers during storage , This study focuses how potato tuber storage fungal infections affect nutritional quality indicators, which is important information for both consumers and the processing industries (Tiwari et al., 2021).

5The main aims of this study :

1- Isolate and identify fungi, from infected potato tuber.

2-Assess their pathogenicity.

2. Literature Review

2.1. Importance of potato

The cultivated potato (*Solanum tuberosum* L.) in South America Originated , the cultivated has been applied for sustenance for more than 10,000 years (Camir et al., 1999). and seven more linked species, which are grown now are growing as the world's most significant non-cereal crop. It is produced in 130 countries on a large scale, with a 2016 global production value of 63.6 billion US dollars (Dolničar and p., 2021).

As the cultivated area reached 2807.75 hectares and the local production for the autumn loop reached 20.9 tons/hectare (Ahmed and sedeeq, 2020). Potatoes are a widely grown, economically significant staple crop that are successfully produced on a large scale, consumed, and are easily accessible in the open market.

4 Basic nutrients found in potatoes include carbohydrates, dietary fibre (skin), potassium, magnesium, and iron, among other vitamins and minerals. (Khalid and humayoun, 2016).

Many different products are made from potatoes after they are processed, such as dehydrated diced potatoes, starch, granules, french fries, chips, cooked potatoes, and defied potato strips (Pedrechi et al., 2016).

2.2. Potato Diseases

Abiotic and biotic stressors, which drastically lower plant productivity, are encountered by plants on a regular basis. Physiological, molecular, and cellular adaptations abound in the complex reactions of plants to these stresses (Ben Rajeb et al., 2014).

This crop has many commercial uses, but a number of soil- or air-borne diseases can result in significant production losses. Approximately 40 soil-borne diseases impact potatoes globally, causing significant harm, particularly to the plant's tubers, which are its most valuable component economically. Soil-borne diseases arise and progress based on a multitude of factors that impact the pathogen or the plant (fiers et al., 2012).

Pests and diseases can be transmitted from one generation to the next, as can vegetatively propagated crops. Significant yield losses were experienced in field and storage conditions due to pathogens such as fungi, bacteria, viruses, and nematodes (Irshad et al., 2014).

One of the biggest biotic barriers to potato production is bacterial illness, which is particularly prevalent in tropical and subtropical areas as well as some warm temperate regions of the world. Worldwide, there are roughly seven bacterial diseases that severely harm potatoes, particularly the tubers, which are the plant's most valuable component economically. The most significant illnesses are believed to be bacterial wilt and back leg, while potato ring rot, pink eye, and common scab are the less serious ones (Charkowski et al., 2020). While potato virus Y (PVY) is one of the oldest plant viruses known to science, it has only become a very serious problem in potatoes in the United States during the last 20 years. The virus is present as a complex of strains that cause a wide range of foliar and tuber symptoms in potatoes, which lowers yield and degrades tuber quality (Karasek et al., 2013).

Potato-associated plant parasitic nematodes consume roots and/or tubers. There have been reports of about 70 species from potatoes, which represent 24 genera. There are no consistent foliar symptoms that indicate nematodes may be the primary cause of stunted growth and decreased tuber yields because they feed on subterranean plant parts. Nematode-damaged potato roots may exhibit lesions, aberrant lateral root proliferation, emerging white females, and brown cysts (Holgado et al., 2012).

One of the most important problems in modern agriculture is the effective and long-term management of fungal pathogens (James et al., 1990).

2.2.1 Fungal Diseases

In most agricultural and horticultural settings, plant fungal infections are the source of disease (Agrios, 2009). The phytopathogens have combined to create strategies and means of attacking any type of plant (Knogge, 1996). actively attempting to gain access and obtain nutrients for development (Horbach et al., 2011). Based on the theory that there are 1.5 million fungal species on Earth, only roughly 70,000 of them have been described, 1.43 million must still be undiscovered. For plant pathologists, the realisation that there are still a great number of undiscovered species is crucial (Hawksworth et al., 1997). Over 8,000 species of fungi and fungal-like organisms (FLOs) have been found to cause disease, making them the group of plant pests that cause the most plant diseases overall (Ellis et al., 2008).

The ability of the plant fungal pathogen to germinate on the surface of an appropriate host is necessary for the occurrence of plant fungal diseases. The spores of the plant fungal pathogen can only sprout under favorable circumstances. This includes the availability of low molecular mass nutrients, a suitable host, and appropriate humidity provided by rain or dew (Osherov and May, 2001). By employing selfinhibitors to halt germination until favorable conditions are met, fungal spores can persist for many (Chitarra et al., 2004).

Plant fungal pathogens create infection structures such as the appressorium and the infection peg when the conditions are right for the hyphae to enter the host (Schfer, 1994).

Based on the mode of infection, plant fungal pathogens can be broadly classified as biotrophs, necrotrophs, or hemibiotrophs. Biotrophs are organisms that feed on living things and infect their hosts without harming them. Some of the pathogens use the appressorium to penetrate the host and feeding structure like the haustoria to source nutrients from the surrounding cells. The range of hosts for biotrophs is restricted, such as rust and powdery mildew fungi (De Silva et al., 2016).

Necrotrophs infect the living host, causing the affected area to eventually die. Necrotrophic fungi can only finish their life cycles on dead tissues, which explains why. Toxins and hydrolytic enzymes are continuously produced by these infections in an effort to kill plant cells. Necrotrophs create two different kinds of toxins called host-specific toxins, which are unique to the plant host and enable the pathogen to infect that host and cause disease (Wen, 2013). Hemi-biotrophs, such as *Colletrichium*, employ similar strategies to biotrophs in order to infect their host and then kill it as a necrotroph (Agrios, 2005).

Phytophthora infestans, Helminthosporium solani, Rhizoctonia solani, Fusarium spp., Alternaria spp., and Colletotrichum coccodes are just a few of the fungal pathogens that can cause postharvest rots on this plant. Marketable yield and potato quality are greatly reduced by rots brought on by infections with these pathogens. Crucially, some of these decaying fungi can also produce mycotoxins, which pose a health risk to people (Tiwari et al., 2020).

2.2.1.1 Late Blight

Potato is an important crop, grown worldwide. It suffers from many pests and diseases among which late blight, caused by the oomycete *Phytophthora infestans*, is the worst. The disease is still causing major damage (Haverkort et al., 2009). Late blight is notoriously known for Irish devastations in the 1840 causing starvation which resulted in the death of 1 million people and mass migrations from Ireland to the USA and other European countries (Zadoks, 2008). Is one of the most threatening pathogenic diseases which not only results in direct crop losses but also causes farmers to embrace huge monetary expenses for disease control and preventive measures (Majeed et al., 2017).

The success of *P. infestans* as a pathogen originates from its ef- fective reproduction in both asexual and sexual forms. In the asexual form, *P. infestans* produces thousands of sporangia per lesion on sporangiophores, which are indeterminate structures that aid in air dispersal of the sporangia using passive movement of wind, rain, or wind-blown rain (Cohen et al., 1997).At lower temperatures, sporangia forgo mycelia growth by directly forming and releasing zoospores (asexual spores),which then germinate and cause new infections at an even more rapid pace (Walker et al., 2007).

Foliage infection causes the premature death of plant which reduces the yield and infected tubers starts rotting in the field and stores (Robertson, 1991). Tubers in the soil become infected by rain-borne sporangia coming from the diseased foliage. Late blight-infected tubers show irregular reddish-brown to purplish areas which extend into the internal tissues of the tubers. The infected tubers usually are hard, dry, and firm but may get attacked by soft rot causing bacteria and rot in the field and stores (Arora et al., 2014).

Classification of the fungi

Kingdom:Straminipila
Phyllum:Oomycota
Class:Oomycetes
Order:Pythiales
Family:Pythiaceae
Genus: *Phytophthora.*

2.2.1.2 Dry Rot

Dry rot is one of the diseases that attack potato tuber, caused by *Fusarium* the disease is commonly found in potato center production areas. The dry root attacking potato tuber is happened at the storage, after harvested. It could decrease a potato tuber yield by 60% (Ren et al., 2021). The disease, caused by several species of *Fusarium* such as *F. solani* var. coeruleum, *F. sambucinum, F. oxysporum, F. avenaceum, F. culmorum*, results in significant yield losses. Some *Fusarium* species associated with the disease produce toxins, which are implicated in mycotoxicoses of humans and animals (Bojanowski et al., 2013). In most dry rot cases more than one *Fusarium* spp. are responsible although reports of a single species infection are rarely noticed (Latus-Zietkiewiez, 1993).

Although dry rot is a severe issue for tubers in storage, also its negative impacts on potato plants appear in the field as wilting and root degeneration (Bojanowski et al., 2013). The wounds formed on the tubers throughout harvesting, handling, and shipping serve as entrance points for fungal spores, and infested tubers induce dry rot in storage. Only when the tuber skin ruptures can the fungus cause infection. The infectious hypha develops in intercellular spaces in living cells and then becomes intracellular only after the cell dies (Xue And Yang, 2020). The spores overwinter from the previous seasons in the soil and remains of decaying tubers can serve as the inoculum source (Al-Mughrabi , 2010). In other cases, the seed tubers and contaminated soils covering the seed tubers can be also the source of spores. During the preparation seed pieces may be infected with spores and start to decay prior to planting in storage and after planting (Wharton et al., 2006). The symptoms of dry rot include sunken and wrinkled brown to black tissue patches on tubers having less dry matter and shriveled flesh. Fungal infection accompanied by toxin development in the rotten tubers raises more concern for consumer health (Tiwari et al., 2020).

Classification of the fungi

Kingdom:Fungi

Phyllum:Ascomycota

Class:Sordariomycetes

Order:Hypocreales

Family:Nectriaceae

Genus:*Fusarium*

2.2.1.3 Early Blight

Early blight, caused by the necrotrophic fungus *Alternaria solani*, is an increasing problem in potato cultivation (Brower et al., 2020).

Alternaria solani is a polycyclic pathogen, because of the possibility of many cycles of infection during a growing season. The primary inoculum produces conidia in the spring. The pathogen overwinters as mycelium or conidia in plant debris, soil, and infected tubers or on other host plants of the same family (van der Waals, 2001). which universally survives in infected leaf or stem tissues on or in the soil where they are grown. Spores form on infested plant debris at the soil surface or on active lesions over alternating wet and dry conditions which are easily carried by air currents, windblown soil, splashing rain, and irrigation water occurring mainly in warm, humid weather with heavy dews or rain (Schultz and French, 2009).

The fungus is carried with the seed, it may attack the seedling, after emergence, and cause damping-off or stem lesions and collar rot. More frequently, however, spores are produced abundantly, especially during heavy dews and frequent rains, and are blown in from infected debris or infected cultivated plants and weeds (Agrios, 2005).The first symptoms appear on older leaves consisting of small, irregular, dark brown to black, dead spots that range from a pinpoint to 1/2 inch in diameter in size. As the spots continue enlarging, concentric rings may form due to irregular growth patterns by the organism in the leaf tissue. This gives the lesion a characteristic "target-spot" or "bull's eye" appearance (Rowe et al., 2021).

Classification of the fungi

Kingdom:Fungi

Phyllum:Ascomycota

Class:Dothideomycetes

Order:Pleosporales

Family:Pleosporaceae

Genus:*Alternaria*

Species: *solani*

2.2.1.4 Black Scurf

Stem canker and black scurf of potato (*Solanum tuberosum L*.) caused by *Rhizoctonia solani* Kühn are important and epidemic diseases in potato-growing regions worldwide (Esfahani, M.N., 2020). This species can cause diseases around 500 plant species (Gush et al., 2019).

Rhizoctonia solani is a soilborne pathogen that is capable of causing seedling damping-off, sheath blight, root rot, collar rot, stem canker, crown rot, bud and fruit rots, and foliage blight on a variety of susceptible agriculturally important crops like soybean, cotton, canola, wheat, beet, potato, rosemary, and turfgrass species (Nagara et al., 2017). During humid summer, stem canker is formed just above ground level and dark brown or black sclerotia can be observed on mature tubers. Infected tubers produced weakened stems and tubers will not grow during sever attack. Rolling of upper leave occur and infected tubers, plant debris and soil provide the place for over wintering (Tsror et al., 2001).

The symptoms of the disease are found on both above and below ground parts of the plant. There are two types of symptoms caused by *R. solani* on potatoes are presence of black colored sclerotia on tubers (black scurf) which is the most obvious sign of Rhizoctonia disease and others occurring as brown, necrotic lesions on stems and stolons below the soil surface (stem canker) (Banville et al., 1996). Other manifestations of infection include poor and uneven stands; premature dying; pruned stolons and sprouts; lesions on roots, stems, and stolons; rosette appearance; girdled stems; necrosis in the stem-end of tubers; russeting of skin; and cracked and malformed tubers (Carling et al., 1999).

Classification of the fungi:

Kingdom:Fungi

Phyllum:Basidiomycota

Class:Agaricomycetes

Order:Cantharellales

Family:Ceratobasidiaceae

Genus:*Rhizoctonia*

Species:*solani*

2.2.1.5 Verticillium Wilt

Verticillium wilt (VW) is a persistent and serious problem in potato production. It is caused by the soil-borne fungi *Verticillium Dahliae* Kleb. in warm production areas and *V. albo- atrum* Reinke & Berthold in cooler regions (Rowe and Powelson, 2002). Both species are soilborne fungi that invade xylem elements, and disrupt water transport in plants and cause vascular wilt in a variety of hosts (Davis et al.,1996). Exudates from the growing root tips of the host enhance micro sclerotial germination, and the resulting high levels of inoculum in the soil favor plant infection (Nagtzaam et al., 1997).

Verticillium wilt is a monocyclic disease, and the pathogen survives between growing seasons as microsclerotia, which also serves as the principal means for pathogen dispersal on seed tubers, farm equipment, and in soil and water (Jansky et al., 2004).

Symptoms of Verticillium wilt of potatoes include unilateral wilting, chlorosis, and necrosis which results in premature senescence of the plant. Symptoms typically occur unilaterally and progress acropetally. Senescent stems often remain upright and contain microsclerotia, the primary resting structures and overwintering inoculum of *V. dahliae*. Reports on the effects of Verticillium wilt on yield are variable, ranging from 12% (Johnson et al., 1986).

Classification of the fungi

Kingdom:Fungi

Phyllum:Ascomycota

Class:Sordariomycetes

Subclass:Hypocreomycetidae

Order:Hypocreales

Family:Incertae sedis

Genus:*Verticillium*

Species:*dahliae*

2.2.1.6 Wart

Potato wart disease, caused by *Synchytrium endobioticum* (Schilb.) Perc., is one of the most dangerous diseases of cultivated potato. *S. endobioticum* is an obligate soil-borne fungus. The pathogen originated in the Andean zones of South America, from which it spread to North America and Europe at the end of the nineteenth century (Przetakiewicz, J., 2015). The disease induces the formation of warts on potato tubers, stolons and stem bases reducing plant growth and yield and making tubers unmarketable (EFSA et al., 2018). *S. endobioticum* is a soil-borne obligate biotrophic organism that does not produce hyphae. Winter (resting) sporangia are the dormant structures by which the fungus disperses to establish new infections. Thick-walled winter sporangia can survive for a long time without plant hosts (Steinmöller et al., 2012).

In response of ward disease, potato plant exhibit rough, warty outgrowths (spherical, spongy,mer and soft) or protuberences on tubers, stolons on the stem, leaves flower and not reported on roots. Resting spores overwinter on infected seed tubers or soil and motile zoospores are spread through potato tubers and soil moisture. Sporangia produced more zoospores which fuse in pairs release in the soil and remain viable for thirty years(Hodgson WA et al., 1974). Warts can vary significantly in size from less than pea-sized proliferations to the size of a fist (Hampson, 1993).

Currently classified as :

Kingdom: Fungi

phylum: Chytridiomycota,

Class: Chytridiomycetes,

Order: Chytridiales,

Family: Synchytriaceae,

Genus: *Synchytrium,*

Species: *Synchytrium endobioticum*

there is strong molecular support for Synchytriaceae to be transferred to the order Synchytriales (van de Vossenberg et al., 2022).

2.2.1.7 Black Dot

Potato black dot is a foliar and tuber blemish disease that has become an increasing economic problem in recent years. The black dot is caused by the fungus *Colletotrichum coccodes* and is characterized by silver/brown lesions on the tuber skin leading to the lower aesthetic quality of potatoes destined for the pre-pack market (Sanzo-Miro et al., 2023). Ubiquitous phytopathogenic fungus with multiple host plants, including weeds and several crops. It is responsible for anthracnose in peppers, tomatoes, and onions, and causes black dot in potatoes (Solanum tuberosum L.) (Aqeel et al., 2008).

In the field, fungal colonization of roots is followed by colonization of stems, stolons, and tubers (Andrivon et al., 1998). Microsclerotia can survive in the soil for long periods, and high soil inoculum levels result in high disease incidence (Lees et al., 2010). and early-maturing cultivars may be less susceptible to the disease because they spend less time in contact with the soil inoculum (Andrivon et al., 1998).

Black dot can affect the yield of potato production (Tsror and hazanovvsky, 1999), and contamination of tubers with *C. coccodes* results in lesions on the skin of potato tubers and water losses during storage (Lees and Hilton, 2003).

The main symptom of potato black dot disease is the presence of macroscopic black sclerotia, the survival fungal structures, on infected tissues (Johnson et al., 2018). Black dot symptoms can be observed in all parts of the plant, and are characterized by the presence of microsclerotia on infected tissue (Read and Hide, 1988).

Classification of the fungi:

Kingdom:Fungi

Phyllum:Ascomycota

Class:Sordariomycetes

Order:Glomerellales

Family:Glomerellaceae

Genus:*Colletotrichum*

Species:*coccodes*

2.2.1.8 Sliver Scurf

Silver scurf is another potato tuber blemish disease, caused by *Helminthosporium solani* Dur. and Mont. Both *C. coccodes* and *H. solani* affect the potato periderm, and even though they are two different pathogens, the symptoms on the potato skin are quite similar , making their detection and differentiation difficult throughout storage (Massana-Codina et al., 2021). and potato is its only known host. Planting infected seed tubers in the field is the primary origin of *H. solani* in the soil (Geary And Johnson, 2006).

However, *H. solani* has been found in decaying material, which, together with volunteer potatoes, can be a source of inoculum (Mérida & Loria, 1994). However, other authors have found that the use of nuclear seed tubers is not sufficient to prevent silver scurf symptoms in progeny tubers, especially in rotations of fewer than 3 years between potato crops (Bains et al., 1996).

As the disease progresses through the potato periderm, black dots turn into silver/brown patches, also known as a black dot lesion , with undefined edges during postharvest storage making the aesthetic impact on potato tubers more significant (Jellis and Taylor, 1974).

Classificationion of the fungi

Kingdom:Fungi

Phylum:Ascomycota

Class:Dothideomycetes

Subclass:Pleosporomycetidae

Order:Pleosporales

Family:Pleosporaceae

Genus:*Helminthosporium*

Species:*solani*

2.2.1.9 Pink rot

Several oomycetes, such as *Phytophthora erythroseptica P. nicotianae,* and *Pythium ultimum*, are known to infect potato tubers, causing pink rot, Phytophthora tuber rot and Pythium leak, respectively (Jahnson et al., 2004).

The potato agro-ecosystem provides a conducive habitat for many foliar and soilborne pathogens. Of these, a number of soilborne oomycetes affect the potato crop causing potential yield, storability, and tuber quality loss (Taylor et al., 2012). Wet and warm conditions promote germination of zoospores in the soil where after the harvesting processes cause wounds or bruises, creating entry points for *P. ultimum* infection post-harvest.(Stine, B.M., 2022).

Phytophthora spp. and *Pythium* spp. differ in mode of infection, where the former is capable of infecting the tuber via stolons, eyes, or wounds, the latter can only gain entry into the tuber through damaged periderm tissue (Salas et al., 2000). left unchecked, water rot pathogens may cause significant tuber yield and quality loss extending from field to storage and storage to transit (Yellareddygari et al., 2016). This disease primarily affects potato tubers, causing a watery breakdown of infected tissue. The tissue may remain relatively firm and rubbery but becomes a distinct pink color after exposure to air for 20 to 30 min. Under conditions favoring severe pink rot, stem and root infections with symptoms similar to blackleg also may occur (Lambert and Salas, 2001).

Classification of the fungi

Kingdom:Straminipila
Phyllum:Oomycota
Class:Oomycetes
Order:Pythiales
Family:Pythiaceae
Genus:*Pythium* ,
Species:*Phytophthora*

2.3 Morphological Identification of fungal pathogen

The diagnosis, treatment, and prevention of fungal diseases have advanced significantly since the publication of Identification of Pathogenic Fungi's first edition. New techniques for diagnosis have been developed (Campbell and Johnson, 2013). Among the most significant creatures that live in the soil are fungi. They are essential for nutrition and other processes that enhance the health and growth of the plant. (Mulani and Turukmane, 2014). Soil fungi are impacted by various factors such as soil properties and human activities, and they are essential in preserving soil fertility and productivity (Bao et al., 2012).

However, a variety of microorganisms, such as yeast and filamentous fungi, live on the surface of plant leaves and fruits, causing deterioration and spoiling of fruits and vegetables. Since plants provide consumers with food and are extremely important to farmers economically, fungi are a top priority. (Udoh et al., 2015). Therefore, the identification and isolation of fungi from various environmental sources remains crucial for the observation and identification of additional species, revision of scientific taxonomy, assessment of their impacts on the environment, and provision of strains for biological control, ecological remediation, and industrial applications (Blackwell, 2011).

The morphology of a fungal colony in filamentous fungi is the result of the hypha, which grows into cylindrical, thread-like structures with diameters ranging from 2 to 10 um and lengths up to several centimeters. Various observations of the colony features, such as color, size, and shape that are visible to the unaided eye, were traditionally used to identify fungi. (Lima and Borba, 2001).

3 . Material and Methods

3.1 Sampling and Isolation of Pure Fungal Cultures

The symptomatic parts were surface sterilized then put on surface of PDA after colonies apear purifid

The study used potato tubers collected from the grocery, which have disease symptoms' and then they were isolated in a laboratory. The tubers were cleaned under the flowing water, and then they were air-dried. The tuber was sliced into a 1 cm size, and then the surface was sterilized by soaking it in sterile distilled water, ethanol 2% of sodium hypochlorite for two minutes. After that, it was washed off with distilled water and then dried on the sterile filter paper. Five potato tuber slices were planted in PDA and incubated for a week. The fungus colony would appear and purify. (Irawati & Asma, 2023).

$$\text{Frequency of isolated fungi } \% = \frac{\text{Colony number of isolated fungi per plate}}{\text{Total no. of colonies per plate}} \times 100$$

Figure (1) Symptoms of potato postharvest diseases collected from local markets

3.2 Identification of Fungal isolates

Macroscopic and Microscopic Examination of Isolated Fungi The fungal morphology was studied macroscopically by observing the colony features (color, shape, size and hyphae), and microscopically by a compound microscope with a digital camera using a lactophenol cotton bluestained slide mounted with a small portion of the mycelium (Gaddeyya et al., 2012).

3.3 Postulate Koch's Assay

Postulate Koch was tested using the agar plug method with slight modification.Sample tubers for testing used the healthy potato tubers . The tubers were cleaned in water. Then they were sterilized by soaking them in sodium Hypochlorite 2% for 10 min. Then they were rinsed three times with sterile distilled water and dried on the kitchen towel before inoculating. Tubers were perforated with a cork borer. About a 0.6 cm depth hole was made for each tube; then, the purified fungi isolate was taken with a cork borer and inserted into the tuber hole. A tuber that had inoculated was put in a plastic container that had a filter on the bottom. Water sparingly was poured at the container base to keep its humidity. Each treatment consisted of 4 replications, in control treatment was potato tubers inoculated by PDA without any fungi.

The mycelium-inoculated tubers were incubated for two weeks at room temperature. Following incubation, potato tubers were cut from the point of inoculation (longitudinally), and the depth of internal necrosis was measured using a ruler. The depth of wound response in controls was also recorded for comparison.

The mean rot volume was calculated using the formula: $V = 1/3\ hr2\ \pi$

where h is the lesion depth and r is the radius (Chen et al., 2020).

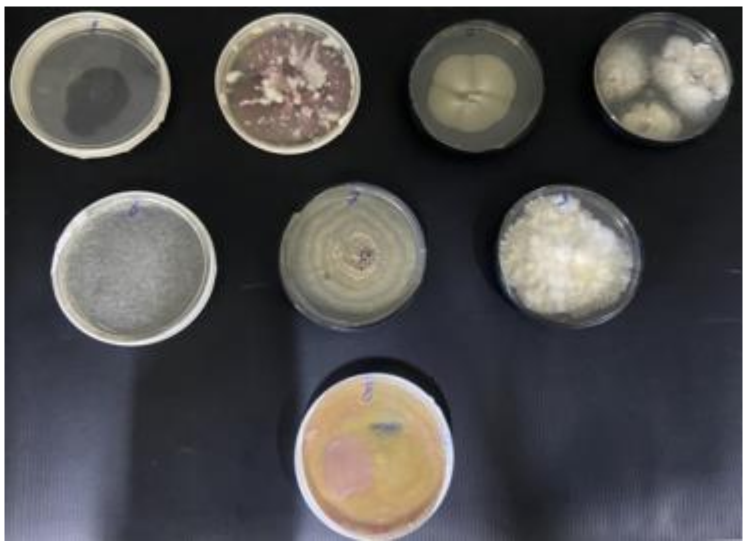
3.4 STATISTICAL ANALYSIS

The data were first checked for normality, and then an analysis of variance (ANOVA) was performed. Duncan's multiple range test was used to compare the means of the parameters (Duncan, 1975)at $p \leq 0.01$ for the vitro experiment by the statistical analysis software IBM SPSS statistics (v28) according to one-way ANOVA (Basto and Pereira, 2012).

4. Results and Discussions

4.1 Fungi associated with potato tubers:

All the samples of potatoes studied were found infested with different fungi. The most common fungi isolated from imported as well as potato tubers were (*Alternaria solani* , *Phytophthora* sp , *Fusarium* sp , *Colletotrichum* sp ,*Clonostachys* sp ,*Rhizopus stolonifer* ,*Rhizoctonia solani* ,*Fusarium solani*)(Figure 2).



(Figure 2) Colonial morphology of fungal isolates used to characterize(1- *Alternaria solani* 2- *Phytophthora* sp 3- *Fusarium* sp 4- *Colletotrichum* sp 5- *Clonostachys* sp

6- *Rhizopus stolonifer* 7- *Rhizoctonia solani* 8- *Fusarium solani*)

The highest percentage of isolated fungi was *Fusarium* sp 26.56% , followed by *Alternaria solani* and *Fusarium solani* 12.5% (figure 3).

In our study, it was observed that *Colletotrichum* sp and *Clonostachys* sp 9.37% . *Phythophthora* sp and *Rhizopus stololonifer* has been reported (6.25, 4.68)% respectively and low frequency appeared by *Rhizoctonia solani* 1.56%.

Similarly, with the previous study (Zehra et al., 2022). was isolated *Alternaria alternata*, *Aspergillusflavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Curvularia lunata*, *Cladosporium sp.*, *Drechslera australiensis*, *Fusarium oxysporum*, *Fusarium solani*, *Mucor sp.*, *Penicillium sp.*, *Phoma sp.*, *Rhizoctonia solani* , *Rhizopus stolonifer*, *Trichoderma harzianum and Trichoderma viride*. From both tuber and potato seed.

The result is similar with (Gashgari and Gherbawy, 2013). was reported *Fusarium, Penicillium, Ilyonectria, Alternaria and Rhizoctonia* were the most common genera. They were represented by *Fusarium oxysporum, Penicillium brevicompactum, P.chrysogenum, Ilyonectria radicicola, Alternaria alternata and Rhizoctonia solani.*

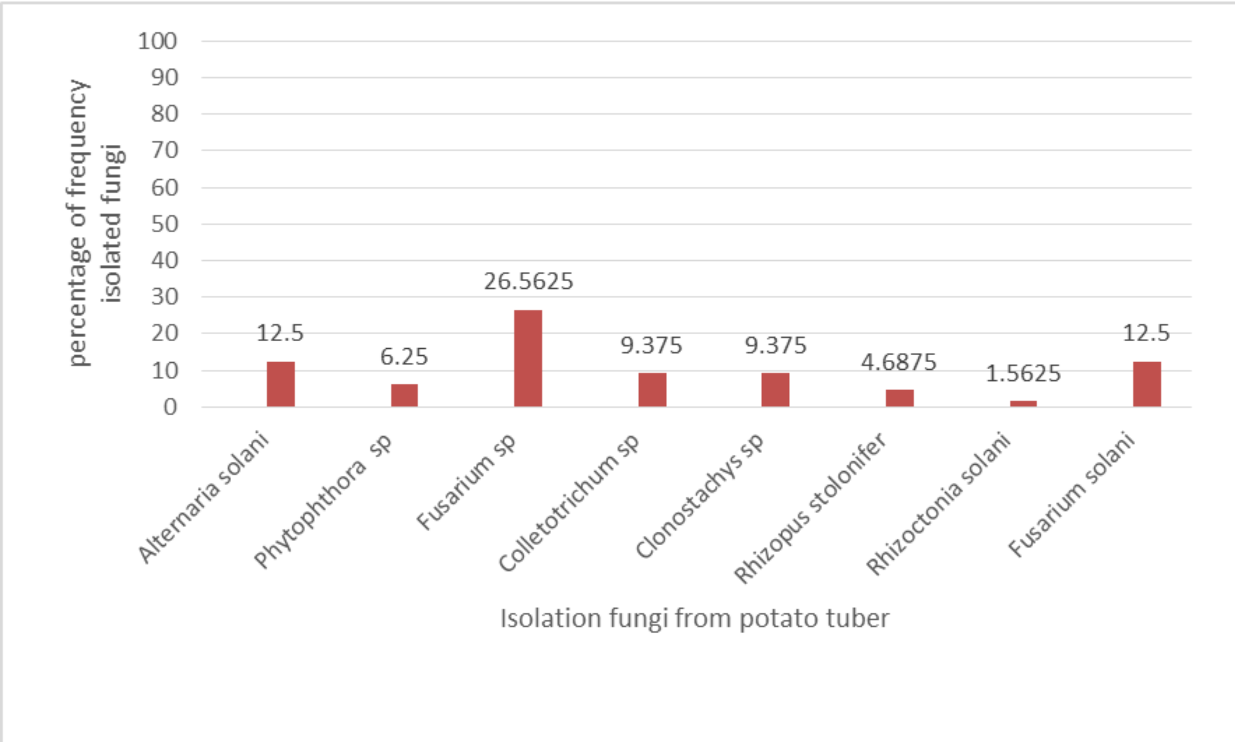
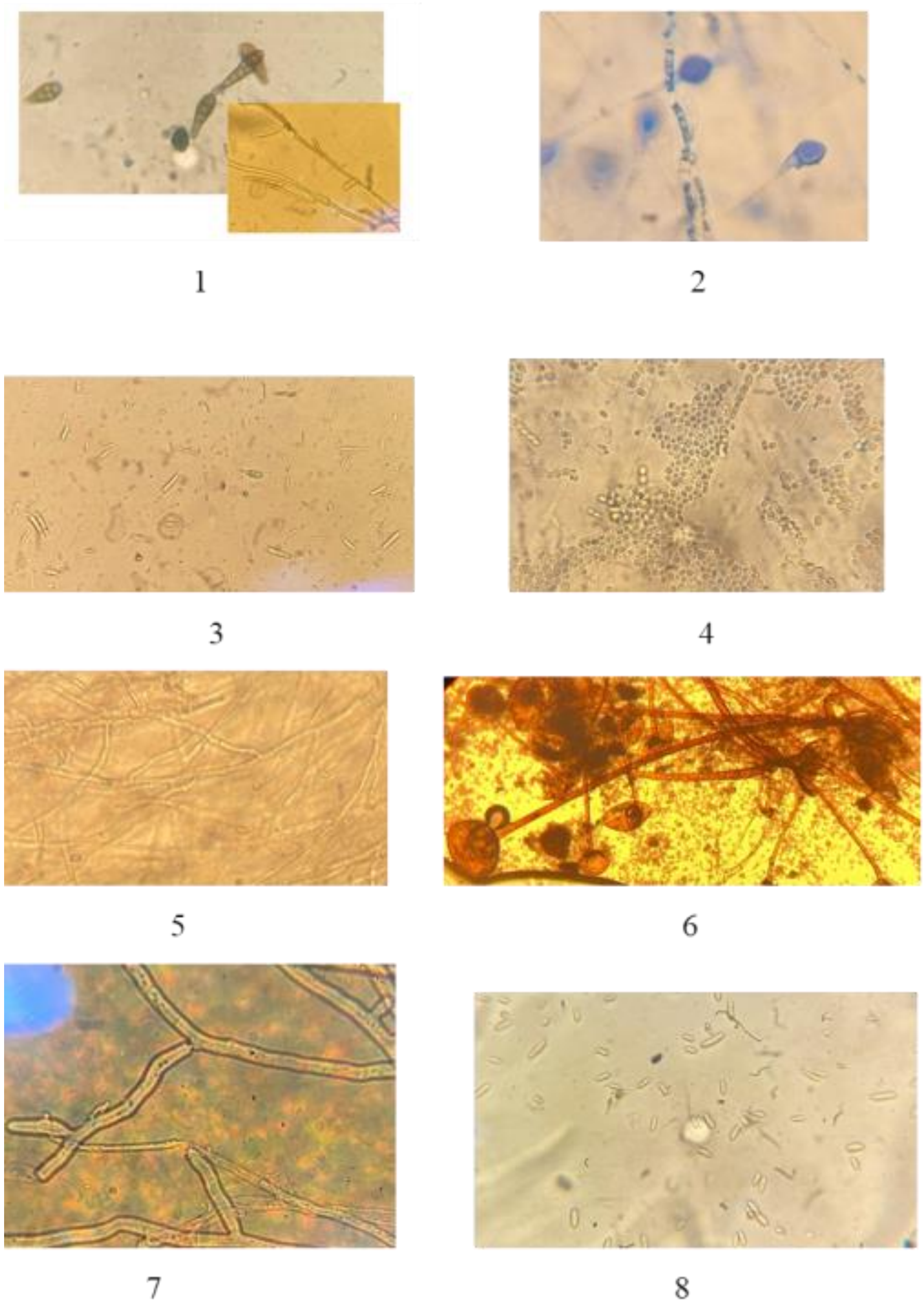


Figure (3) Percentage of frequency Fungai isolated from the potato tubers

4.2 Macroscopic and Microscopic Features Isolated Fungi

In this study, the isolated fungi were examined based on cultural, (color, shape, size, and hyphae), microscopic, and morphological characteristics. (Figure 4).



(Figure 4) Morphological characteristics of some selected parts producing fungi.

(1- *Alternaria solani* 2- *Phytophthora* sp 3- *Fusarium* sp 4- *Colletotrichum* sp 5- *Clonostachys* sp 6- *Rhizopus stolonifer* 7- *Rhizoctonia solani* 8- *Fusarium solani*)

4.3 Postulate Koch's Assay

Table (1) showed The result of postulates Koch's showed that rot symptoms appeared on the potato inoculated by different isolated fungi The symptom area had a different symptom volume.

It had Significant differences among the isolated fungi the biggest rot volume by *Fusarium solani* was (360.71) mm followed by *Alternaria solani* records rot volume (162.36) mm. The lowest rot volume by *Colletotrichum* sp and *Rhizopus stolonifer* (54.82,45.79)mm respectively (figure 5).

The results are similar to both previous studies by (Ramadan and Haleem (2023) and Irawati and Sembiring, (2022) In Koch's Postulate test, and similarity to the study (Falert and Akarapisan, 2019). of The effect of wounding on infection by Fusarium species in potato tubers was studied, four injury levels were evaluated to determine the influence of wound severity on infection by each Fusarium species. isolates showed necrotic symptoms (rot volume) with varying necrotic areas.

Table 1. Rot volume as measured by mean depth of necrosis (in mm) by koch's postulate of 8 isolates following wound inoculation of potato tubers.

Isolation fungi	Rot volume	Pathogenicity rating
Control	25.12^{e*}	NP
Alternaria solani	162.36^{bc}	P
Phytophthora sp	97.99^{bcd}	P
Fusarium sp	62.08^{cde}	P
Colletotrichum sp	54.82^{cde}	P
Clonostachy sp	123.44^{bc}	P
Rhizopus stolonifer	45.79^{de}	P
Rhizoctonia solani	116.83^{bc}	P
Fusarium solani	360.71^{a}	P

*Means had the same letter or letters are no-significantly different at $p \leq 0.01$ a according to Duncan

(Figure5) Potato tubers infected with (Colonial morphology of fungal isolates used to characterize(1- *Alternaria solani* 2- *Phytophthora* sp 3- *Fusarium* sp 4- *Colletotrichum* sp 5- *Clonostachys* sp 6- *Rhizopus stolonifer* 7- *Rhizoctonia solani* 8- *Fusarium solani*)

5-Conclusions

1- Eight genera of fungi (*Alternaria solani* , *Phytophthora* sp , *Fusarium* sp , *Colletotrichum* sp , *Clonostachys* sp , *Rhizopus stolonifer* ,*Rhizoctonia solani* and Fusarium solani) were associated with potato tubers.

2- The highest isolation frequency of fungi *Fusarium* sp while *Rhizoctonia solani* was the lowest isolation frequency.

3- A significant effect of the pathogens was detected rot volume. the biggest rot volume *Fusarium solani*, the lowest volume rot symptom of *Colletotrichum sp* and *Rhizopus stolonifera*.

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