WOLKITE UNIVERSITY

COLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

DEPARTMENT OF BIOTECHNOLOGY

ISOLATION AND CHARACTERIZATION OF FUNGI ASSOCIATED WITH SPOILAGE OF TOMATOES IN GUBRE MARKET

SENIOR RESEARCH

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A Senior Research Project Submitted to Department of Biotechnology in Partial Fulfilment of the Requirements for the Bachelor of Science Degree in Biotechnology

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LIST OF ABBREVIATIONS

Sps……species

PDA……Potato Dextrose Agar

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ABSTRACT

Tomato contains large amount of water which makes it more susceptible to spoilage by fungi. These fungi are detrimental to human health, this study was therefore carried out to isolate, characterize and identify the fungi species in spoiled tomatoes. Tomato fruits sold in different areas in gubre, wolkite were used for this study. Thus it was found that fungi were the source of spoilage in most of the samples rather than bacteria. Further morphological studies were done to determine the fungal strains responsible for the spoilage. Among the fungi, it was found that Aspergillus niger and Rhizopus stolonifera were found in most of the spoiled samples with a few samples with A. niger dominating all the plates Pathogenic test result showed that Aspergillus niger had the highest in health tomato fruits while Rhizopus stolonifera had the lowest. Proper handling and adequate storage, facilities must therefore be employed to prolong the shelf life of tomato fruits.

Keywords*: Tomato, Fungi, characterization, spoilage, Pathogenicity test.*

1. INTRODUCTION

1.1 Background of the Study

Tomato (*Solanum lycopersicum L.*) belongs to the family solanaceae and it is an annual sub-tropical vegetable fruit crop. The crop originated from South America and was introduced to Europe in the 16th.The determination of fungi associated with the spoilage of tomatoes involves a series of steps to identify and characterize the fungal species responsible for the deterioration of the tomatoes.Tomatoes (*Solanum lycopersicum*) are widely consumed and constitute a significant component of human diets due to their nutritional value and culinary versatility. Fungal contamination not only leads to economic losses in the tomato supply chain but also poses a threat to food safety and public health. Moreover, fungi affecting Tomatoes includes *Fusarium oxyspanium, Fusarium stolonifer, Aspergillus niger and Rhizopus stolonifer (*Mallek*, et al,*.1995).

Fungi are diverse microorganisms that thrive in various environmental conditions, and some species have adapted to exploit the unique characteristics of tomatoes as a substrate for growth. The spoilage of tomatoes by fungi is a complex process influenced by factors such as moisture content, temperature, storage conditions, and the presence of wounds or lesions on the fruit. Understanding the specific fungi associated with tomato spoilage is crucial for implementing effective control measures and ensuring the quality and safety of tomato products.

1.2 Statement of the Problem

Tomatoes are a highly perishable crop and are susceptible to spoilage by various microorganisms, including fungi. In Gubre, tomato production is a key component of the agricultural sector, but the country experiences significant losses due to spoilage during harvest, transportation, and storage. Spoiled tomatoes not only result in economic losses for farmers and distributors but also pose a threat to food safety and public health.

Identification and characterization of the specific fungal species responsible for tomato spoilage are crucial for developing effective control measures and ensuring the quality and safety of

tomato products. Therefore, this study aims to isolate and identify the fungi associated with spoiled tomatoes in Gubre market and determine their prevalence and pathogenicity. By addressing the problem of fungal spoilage in tomatoes, this study will contribute to improving food safety and reducing economic losses in the tomato supply chain.

1.3. Objectives of the study

1.3.1 General Objective

(i) To Isolate and Identify of Fungi Associated with Spoiled Tomatoes in Gubre

1.3.2 Specific Objective

(i) To isolates fungi species associated with spoiled tomatoes in gubre.
(ii) To characterize and identify fungi species associated with spoiled tomatoes.
(iii)To determine the pathogenicity of fungal organisms.

1.4 Significance of the study

Because of poor management and high water content of tomato is spoil with several fungi species so knowing that main spoilage causing species of fungi and fixing these problem.

In Addition to this, it is better to separate spoiled tomato from the fresh (that is not spoil with fungi). This study is significant as it identifies the key fungal species responsible for tomato spoilage in Gubre market, contributing to a better understanding of the factors leading to microbial decay and deterioration of tomatoes. This knowledge will enable the development and implementation of targeted control measures to prevent spoilage, ensuring the availability of high-quality tomatoes for consumers and reducing economic losses for farmers and distributors. Additionally, fungal spoilage not only affects the physical appearance and taste of tomatoes but can also produce mycotoxins, which pose health risks to consumers when consumed.

Thus, by characterizing and identifying the fungi causing tomato spoilage, this study assesses the potential health hazards associated with contaminated tomatoes and guide the implementation of appropriate safety regulations. Lastly, addressing the issue of fungal spoilage in tomatoes contributes to overall economic development.

2. LITERATURE REVIEW

2.1 History of Tomato

Tomato (*Solanum lycopersicum L. formerly Lycopersicon esculentum Mill.*) is a new world crop with its centre of origin in Peru and surrounding countries in South America. Its domestication may have occurred independently in Peru and Mexico, although the evidence is not conclusive (Peralta *et al,* 2007).

Evidence suggests the Mexican domestication had occurred by 1523. Spaniards took it to Europe, with the first references of its cultivation and uses in 1544 European immigrants took the tomato to North Americ a in the 1600s, but superstitions as to it being poisonous limited its use(Caicedo *et al* 2013).

2.2 Nutritional Facts of Tomatoes

Tomato contains large amount of water which makes it more susceptible to spoilage by fungi. These fungi that spoil tomatoes produce mycotoxins that are detrimental to human health.Tomato fruits have high dietary and nutritional qualities. Tomatoes are essential mainly for its dietary needs and can be consumed in diverse ways. It is rich in nutrients,vitamin, dietary fibres and phytochemical (Bradley, 2003; Freeman and Reimers,2011; Mariga, 2012). Tomatoes has a much lower sugar content than other fruits and is therefore not as sweet and their low pH values make them particularly desirable to fungal decay.The importance of tomatoes in the food industry and its nutritional benefits cannot be over emphasized.

Routine microbiological examination of tomatoes is very crucial as it contributes to a large extent to economic development. Post harvest handling, weak storage practices, transportation and improper marketing are seriously affecting the quality of tomatoes. Tomato fruit rots are mainly caused by fungi. Several fungal species were associated cause of fungal infections in tomatoes.

2.3 Causes of Tomato Spoilage

Spoilage of tomatoes are those adverse changes in the quality of tomatoes that are brought about by the action of predominantly biological and physical factors. These may be changes in taste, small, appearance or texture of the fruit. (Ghosh, 2009).Their spoilage by fungi results in loss of economic resources as well as food poisoning.

The fruits are usually transported from areas of production to areas of consumption in locally woven baskets and snacks under conditions that encourage the growth of fungi. Both biological and physical damage during the harvest and transportation phase, coupled with the large amount of water and soft endocarp more This fruit are usually displayed on benches and in baskets for prospective customers in the open markets until sold there by exposing them to further microbial infection beside those associated with these whole fruit surface and those from adjacent infected fruits (Baijewu *et al*., 2007)

Tomato contaminated with Fusarium species is dangerous for human health, because they produce mycotoxins (Nelson *et al*.,1990). Alternaria rot has been considered common diseases and cause huge losses to tomatoes thus making tomatoes unfit for consumption (Douglas, 1992).

2.4 Control and Preservation of Tomatoes

Tomatoes are highly perishable so it is usually consumed immediately after harvest. In the event that consumption does not take place after harvest there is need to preserve so that spoilage does not occur. Good quality control measures must be employed by the farmers, marketers and consumers during the harvesting, transportation, handling and processing of the fruits.Supremely ripe and juicy tomatoes can be preserved by canning, freezing and drying. these methods are time consuming but none required special skills or anything complicated (Addison, 2014).

3. MATERIALS AND METHODS

3.1 Study area

This study was conducted at the Department of Biotechnology, Wolkite University, Wolkite. Wolkite is found in centeral Ethiopia, located 165 kms from Addis Ababa. The administrative center of the Gurage zone of the Central Ethiopia. This town has elevation between 1910 and 1935meters above sea level.

3.2 Sample Collection

A systematic random sampling approach was used to collect the tomato samples from Gubrye market using hand gloves, and particular attention was given to selecting spoiled tomatoes for the study. The samples was transported to the laboratory in sterile polythene bags for further analysis. All the glasswares that used for the experiment was properly washed, dried and sterilized in the Autoclave at 121^0C, 15psi,15min. The entire working surfaces was disinfected with 70% ethanol to eliminate contaminants.

3.3 Sample Processing

Upon arrival at the laboratory, the spoiled tomato samples was processed by carefully cutting and aseptically removing a thin slice sections of the rotten parts using a sterile scalpel. Sections of the sliced tomatoes where homogenized using a stirring rod, and five fold serial dilution was prepared.

3.4 Isolation of Fungi

For the isolation of fungi, the pour plate method using sterile Potato Dextrose Agar (PDA) was employed. The contents were properly mixed and dispensed aseptically into Incubation; after that carried out in an inverted position at 28^0C for five days. The colonies that developed were counted and subcultured repeatedly on potato dextrose agar plates to obtain pure cultures. They were later stored on PDA slants for characterization and identification.

3.5 Purification of fungal isolates:

During the 3days of incubation, plates were observed for obvious growth and plate that displayed growth are marked and sub-cultured to obtain pure colonies. After obtaining the pure colonies, they were preserved in sterile bottle for further use. The stock bottle was kept in a refrigerator at 4^0C.

3.6 Pathogenicity Test of the Isolates

To assess the pathogenicity of the isolated fungal species, a pathogenicity test conducted by inoculating healthy tomato fruits with the isolated fungi. The inoculated tomato fruits and the control placed in the incubator at 28^0c for 3 days and observed for spoilage. The decay rate of each fungus in the healthy fruits also determined by measuring its rot diameter after three days of its inoculation into the healthy tomato fruit. Pathogenic microorganisms on tomato are a potential health hazard to man and animals following ingestion.

4. RESULT

4.1 Sample Collection and Frequency of Fungal Isolates

Tomato samples exhibiting spoilage symptoms were collected from Gubre Market. A total of four samples were randomly selected and brought to the laboratory for further analysis. The fungi were isolated using standard microbiological techniques, including surface sterilization and plating on Potato Dextrose Agar (PDA). Out of the four tomato samples, *Aspergillus niger* and *Rhizopus stolonifera* were the observed fungal growth. A total of two fungal isolates were selected for further characterization.

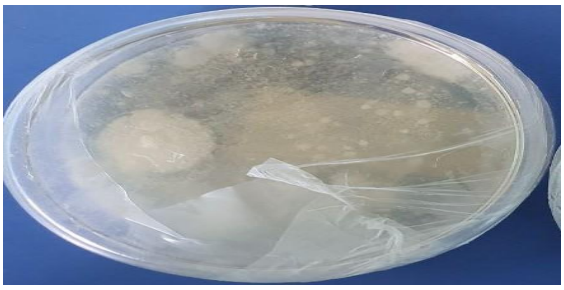
4.2 Phenotypic Characteristics of Fungal Isolates

4.2.1 Morphological Features

The phenotypic characteristics of the fungal isolates were determined based on colony morphology and pigmentation on PDA. *Aspergillus niger's* **is** **c**olonies appeared black with a powdery texture. The *A. niger* was characterized by morphology characteristics are rapid growth on the PDA media and its **c**olonies appeared black with a powdery texture with white mycelia in addition to the that the shape of the colony was thin. The colony texture was distinguished by having distinct and clear growth areas and it has formed a powdery texture as a result of heavy sporulation. The edges of the colony were surrounded by white mycelia while the reverse side was colorless to pale yellow and surrounded by mycelia in the form of a white circle.

All of these macroscopic features are illustrated in Figure 1 (A,B,C)

A. sample 

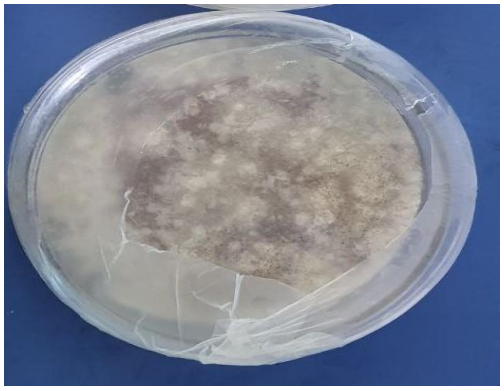
B. Culture 

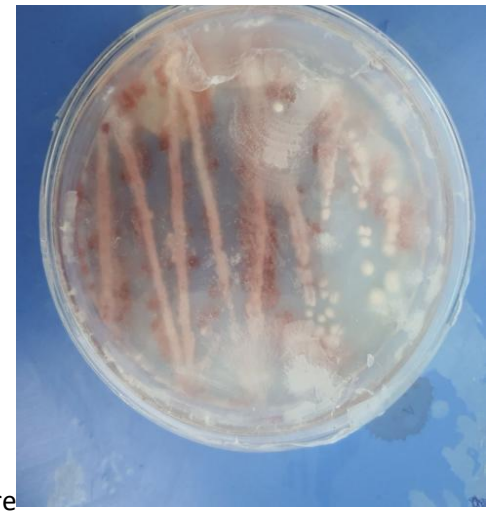
C. Sub culure 

Rhizopus stolonifera colonies were fast-growing, white initially, turning grayish black,dense fluffy growth, reverse is gray or brown.

The pathogen first appear as white cottony colonies on potato dextrose-agar at 25°C becoming heavily speckled by the presence of sporangia and then brownish black in age.

Figure 2 (A,B)

A. culture 

B. sub culture 

4.2.2 Pathogenicity Test

The pathogenicity test was conducted to confirm the ability of the isolated fungi to cause spoilage in tomatoes. Healthy tomatoes were inoculated with spore suspensions of each fungal species and incubated at room temperature for 7 days. All the tested fungi caused spoilage symptoms, including soft rot, discoloration, and mold growth, confirming their pathogenic nature.

5. DISCUSSION

The fungi associated with the spoilage of tomato fruits sold in major markets in gubrye was studied and the result revealed the presence of a teeming population of fungi. It could be seen that, *Aspergillus niger* and *Rhizopus stolonifer* were the fungal species isolated and identified predominant among the samples. Based on the research performed and the observation achieved, the fungal species likely to be available in all the spoiled tomatoes are mainly but bacterial species was inoculated on PDA Culture medium because ther was lack of antibiotics wich prevent bacterial growth. The percentage occurrence of the fungi in relation to the markets showed that the fungi had the highest percentage occurrence of in the samples from gubrye market.

The fungal isolates from the fruits were Aspergillus niger and Rhizopus stolonifer (Ibrahim et al,.2011) found Aspergillus niger as one of the major fungi responsible for the production of volatile compounds in spoilt tomatoes as we looked at size for its appearance. (Baker,et al,.2006) also found Aspergillus niger from rotten tomato fruits and reported that they are pathogenic on tomato fruits. (Akinmusire, et al,. 2011) reported that Rhizopus spp were associated with the spoilage of tomatoes. (Wogu and Ofuase,et al,.2014) found Aspegillius spp, Penicillum spp, Fusarium spp and Saccharomyces spp from spoilt tomato fruits. (Mbajiuka and Enya,et al,. 2014) also isolated Aspergillius spp, Penicillum spp and Saccharomyces cerevisiae from spoilt tomatoes. (Ghosh,et al,.2009) also found Aspergillius niger and Rhizopus stolonifer from the spoilt tomato fruits studied.

These fungi isolated in this study are sources of potent mycotoxins which are detrimental to health though their consumption. Ochratoxin mycotoxin which is considered to be a potential Carcinogen, give rise to nephrotoxicity and renal tumour.Farmers and marketers of the produce are also advised to take appropriate precautions during the harvesting, transportation, storage and sale of tomatoes to reduce the risk of these toxins and other metabolites that are deleterious to health.

6. CONCLUSSION

Isolating and characterizing fungi associated with tomato spoilage provides essential insights into the fungal species involved, their spoilage mechanisms, and the environmental factors that favor their growth. The present study observed that many fungal pathogens are associated with tomato diseases. In present investigation, *Aspergillus niger*, *Rhizopus sp*, are found to be major disease causing organisms. *Aspergillus niger* was appeared to be most active of all the pathogens that result losses of economic resources as well as mycotoxins. Control measures must be employed by vegetable growers, marketers and consumers at the time of harvesting, transportation, handling, storage and processing of tomato fruit. This needs critical steps in understanding the factors contributing to post-harvest losses.This knowledge is crucial for developing targeted strategies to prevent spoilage, ensuring food safety, and minimizing economic losses.The identified fungi are characterized by their ability to produce enzymes and toxins that degrade tomato tissues, leading to spoilage. Understanding these mechanisms helps in developing strategies to mitigate fungal growth.

7. RECOMMENDATION

It is recommended that tomato fruits production should be handled with extreme care. Good quality control measures must therefore be employed by the farmers, marketers of the fruits. Frequent inspection of fruits for sales by food inspectors is also recommended. These will go a long way in preventing the consumption of contaminated tomato fruits thereby reducing the health hazards posed by the mycotoxins produced by these fungi isolated in this study.Washing the tomato with clean water before consumption will also reduce the fungal load.

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