Fungi Isolation and identification associated with postharvest spoilage of fruits

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Abstract— According to annual studies, 20% of the produce of fruits and vegetables spoils. This study explores certain botanical extracts as potential alternatives to industrial chemicals in an effort to address the problems associated with fruit postharvest losses. Step-by-step instructions wereused to complete this work. The materials utilised for the analysis were sanitised, and samples of rotten fruits were randomly gathered. Pathogens were subsequently isolated at the Microbiology Laboratory. Following isolation, the pathogens were also identified. By use of the culture process, the fungus was isolated, and their morphological traits were used to identifythem. We looked at how extracts of tulsi leaves, neem leaves, turmeric bulb, and garlic bulbs affected the proliferation of isolated fungi. Preparation of crude extracts of turmeric bulb, neemleaves, tulsi leaves and garlic bulb and determination of the efficacy of these extracts on identified pathogens were conducted. Anti -fungal activity against the isolated fungi is checkedusing the natural components like tulsi leaves, neem leaves, turmeric bulb and garlic bulb. Theidentified fungi were *Aspergillus fumigatus, Aspergillus terreus, Aspergillus flavus*. Among the different natural components used containing antifungal activity, garlic was the most efficient and gave the best results. Garlic was most prominent in treating fungal infection caused by Aspergillus flavus.

Keywords: Fruits, fungi, isolation, pathogens, spoilage

I. INTRODUCTION

Fruits, which are often consumed uncooked, are the edible portion of the mature ovary of flowering plants [1]. Many structures that are popularly referred to as fruits, such as bean pods,corn kernels, tomatoes, and wheat grains, are also considered to be fruits [2]. The growth and presence of fungi in food can lead to food deterioration, which lowers both the quality and quantity of the food [3]. The therapeutic and antibacterial properties of plant extracts are attracting the attention of scientists all around the world. Modern medicine has its own benefitsand drawbacks, thus plant-based treatments are becoming more and more popular because theyare safe to use, relatively simple to get, and inexpensive. Numerous substances have antifungalproperties [4]. Fruit plays a crucial role in human nutrition since it contains vital growth components like vitamins and minerals required for healthy body metabolism [5]. Fruits havebecome a staple meal for both humans and many animals [6]. Fruits, however, are prone to spoilage and typically have an active metabolism when in storage [7]. The successful growth and survival of numerous parasitic and saprophytic species of fungi is also aided by the low pH and high concentration of certain minerals, vitamins, carbohydrates, and amino acids [8]. According to annual studies, 20% of the produce of fruits and vegetables is lost to spoiling, particularly during the post-harvest stages. This has been linked to fungi that can be toxic or pathogenic known as spoiling fungi [9]. Fruits include high levels of iron, calcium, phosphorus,magnesium, copper, niacin, iron, folate, vitamins A, B6, E, and C, as well as carbs [10]. In addition to their use in medicine, plant-based products are increasingly being used for plant protection in countries all over the world [11].

I.I. GARLIC

One of the most significant and anciently recognized plants for medicine is garlic. There has alengthy history of its use everywhere [12]. Allium sativum L., also known as garlic, is a member of the Liliaceae family and is categorized with onions [13]. Being a vital culinary spice plant, garlic plays a crucial role in disease prevention and control, and it can treat many illnesses [14]. It has long been used to combat human infections. Studies on the use of garlic to combat plant diseases, however, are scarce. Some earlier research discuss the pathogen- fighting capabilities of garlic [15].

I.II. TULSI LEAVES

Tulsi, scientific name Ocimum gratissimum, is revered and regarded as a sacred plant in India.It's a member of the Lamiaceae family. It is a beneficial medicinal plant with many differen pharmacological attributes [16]. Ocimum sanctum (Family Labiatae) is a 75 cm tall, numerously branched, upright, sturdy, and scented herb. This tiny plant can be found all over India and is grown there as well as worshipped in Hindu temples and homes. This is also knownas India's Holy Basil in English, Vishnu-Priya, Tulsi in Sanskrit, and KalaTulsi in Hindi. Thisplant's roots, seeds, and leaves have all been employed in traditional Ayurvedic treatment [17].

I.III. TURMERIC

Curcuma longa L., also known as turmeric and haldi in India, has anti-oxidant, anti- inflammatory, antiviral, anti-bacterial, and antifungal effects. Herbalists are investigating the possibility of this plant in treating the COVID-19 pandemic [18]. In India, China, and South East Asia, turmeric (Curcuma longa) is widely used as a spice, food preservative, and colouringagent. Turmeric has a long history of usage in traditional medicine around the world in additionto its culinary applications [19]. The primary yellow bioactive component of turmeric, curcumin (diferuloylmethane), has been found to have a variety of biological effects. It was also frequently used to enhance intestinal flora, aid with digestion, and heal skin irritations [20].

I.IV. NEEM

Neem (Azadirachta indica), sometimes known as "Indian Lilac," is a member of the tribe Melieae and the families Meliaceae and Meloideae. Neem is one of the most adaptable, diverse,and capable tropical trees. More than any other tree species, it has the most beneficial non- wood products (leaves, bark, blossoms, fruits, seeds, gum, oil, and neem cake). These non- wood products are known for their biological properties, which include those that are anti- allergic, anti-dermonic, anti-feedent, anti-fungal, anti-inflammatory, anti-pyorrhoeic, anti-scabic, cardiac, diuretic, insecticidal, larvicidal, nematicidal, and spermicidal. Neem has a widerange of uses as a result of these actions, making it a green gem [21].

II. MATERIALS AND METHOD

II.I. PREPARATION OF FUNGAL STRAIN

A fruit (tomato and guava) was randomly selected for isolation of fungus. The collected fruitswere kept in an air tight closed container to spoil for few days (directly spoilt fruit can be collected from the market). The spoilt fruit is then used for isolation of fungi. As observed thetomato and guava kept is spoilt and visible growth of organism can be seen. A small part of thefruit is cut from the corner with the skin intact. Then this small infected part in kept in the middle of the prepared Sabouraud Dextrose Agar (SDA). Then the plates was inserted into anincubator to obtaining the growth for 24-48 hours. The mixed growth of fungi is obtained by using rotten fruits. To obtain pure culture the fungus is subcultured few times [22].

II.II. PREPARATION OF DISC

Whatman filter paper is collected from the lab. The Whatman filter paper, with the help of puncher is punched to obtain disc of same diameter (size: 6mm). This paper is the kept in the sterilized plate. Then kept in the hot air oven for 20 min to obtain sterilized disc so that contamination doesn't occur [23].

III. FUNGAL IDENTIFICATION

LACTOPHENOL COTTON BLUE STAINING:

To Lactophenol Cotton Blue works on the principle of staining the fungal cell walls for the identification. The fungal spore cell wall is made up of chitin, which is stained by LactophenolCotton Blue for identification.

III.I. PLANT SAMPLE MATERIAL PREPARATION

The samples were collected from nearby markets. The collected samples are then washed throughout with distilled water are dried. With the help of mortar and pestle the leaves are theleaves are grinded till a paste like texture is obtained. After some time of grinding the mixturewill be thoroughly mixed. The mixing is done without adding any additives or water. Immediately, juice was filtered using muslin cloth. Then the disc prepared using the Whatmanfilter paper is places using sterile forceps. Wait for some time till the Whatman filter paper is completely soaked in the mixture. The filter paper is then placed on the agar plate containing the fungi to check the antifungal activity of Tulsi against the fungus. This plate is then incubated at 25°C for 24-36 hours.

III.II. ANTIFUNGAL SENSITIVITY TEST

The antifungal activity of fresh garlic juice, turmeric juice, neem juice and tulsi juice was determined using Kirby-Bauer disc diffusion method as reported in **Abdallah, et al** with minormodification [23]. 20 ml of Sabouraud dextrose agar was autoclaved, heated, and then placed hot onto sterile Petri plates (90 mm in diameter), where it was allowed to cool and solidify. Then, using sterile cotton swaps, 100 µl of each fungal strain from the previously prepared broth cultures was decanted over the agar plates. Discs saturated with garlic juice, turmeric juice, neem juice and tulsi juice was placed on the seeded agar plates, then incubated at 25°C for 48-72 hours. After incubation, the diameter of the clear zone of inhibition around the discswas measured in millimetre (mm)

IV. RESULT AND DISCUSSION

Mixed culture was obtained, were subcultured to obtain pure culture and by various analysis results the obtained fungi were of *Aspergillus fumigatus, Aspergillus terreus* and *Aspergillus flavus.*

Figure 1. ***Aspergillus flavus***

Figure 2. ***Aspergillus fumigatus***

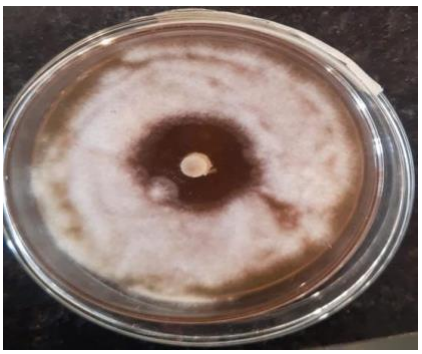


Figure 3. *Aspergillus terreus*

Natural antifungal containing plant components like garlic, neem, tulsi and turmeric were tested against these fungi to determine their antifungal activity.

Interestingly, Aspergillus fumigatus, Aspergillus terreus, Aspergillus flavus were highly susceptible to garlic juice in comparison with the any other natural components containing antifungal activity. The values of antifungal activity of tested fungi are tabulated in Table 1 and expressed in bar-graph as shown in graph 1.

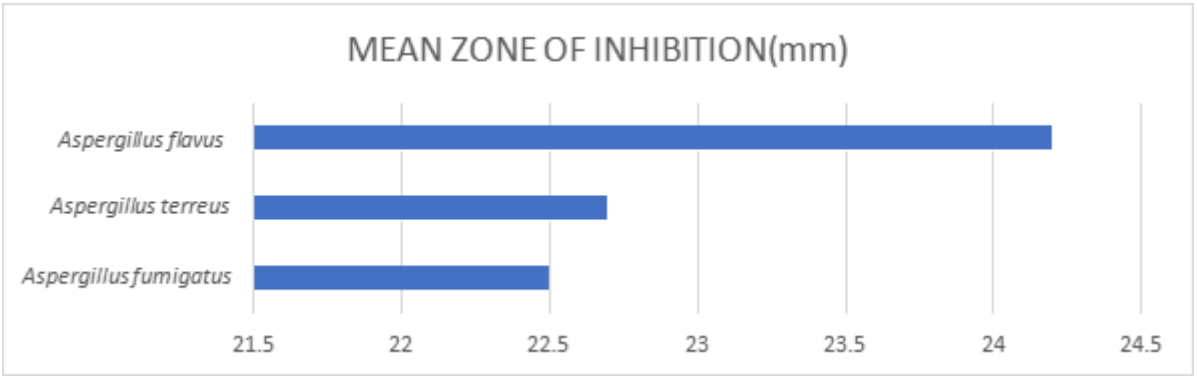
The mean zone of inhibition of Aspergillus was fumigatus 22.5±1.5 mm with garlic extract butwas next to negligible in juice extract of Tulsi, Neem and Turmeric.

The mean zone of inhibition of Aspergillus terreus was 22.7±1.6 mm with garlic extract but wasnext to negligible in juice extract of Tulsi, Neem and Turmeric.

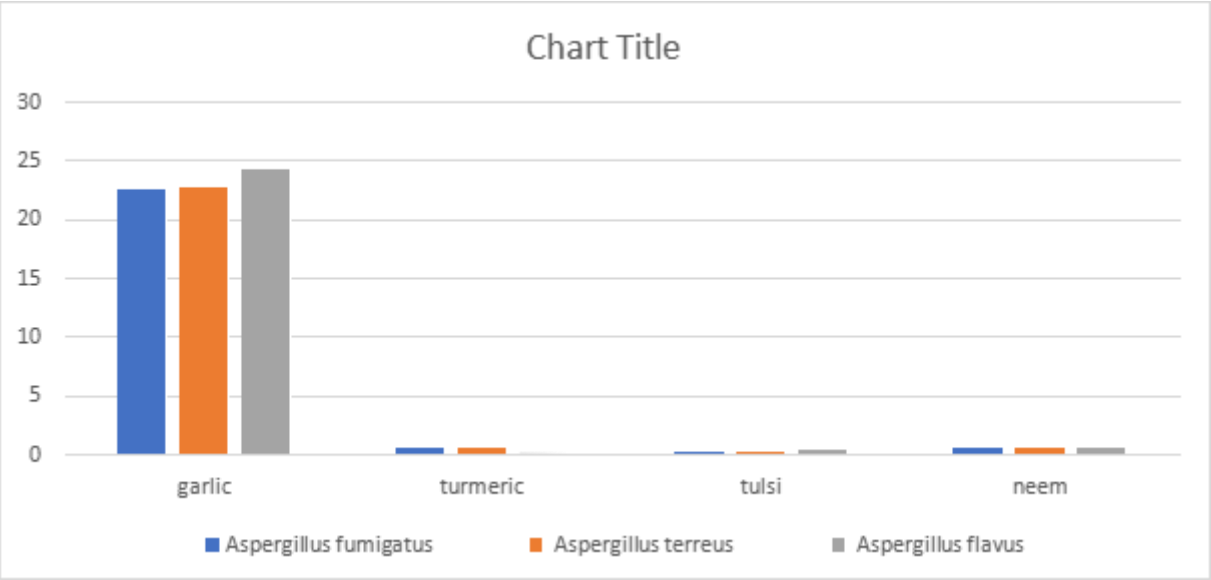
The mean zone of inhibition of Aspergillus flavus was 24.2±1.6 mm with garlic extract but wasnext to negligible in juice extract of Tulsi, Neem and Turmeric. The statistical analysis showedthat when to compare the antifungal efficacy of garlic, Tulsi, Neem and Turmeric, garlic as antifungal was statistically significant as shown in graph 2.

Table 1. Antifungal Activity of Garlic extract

FUNGUS STRAIN	MEAN ZONE OF INHIBITION (mm)
Aspergillus fumigatus	*22.5±1.5 mm*
Aspergillus terreus	*22.7±1.6 mm*
Aspergillus flavus	*24.2±1.6 mm*



Graph 1. Mean zone of inhibition of Garlic extract



Graph 2. Mean zone of inhibition

All the fungal strains of *Aspergillus* fumigates, *Fusarium laceratum, Geotrichum candidum, and Trichoderma hamatum* were susceptible to garlic, according to Rees, et al., citing numerous earlier investigations that support the current findings [24].

The antifungal potential of garlic was also assessed against a few post-harvest fungal pathogens, including *Neofabraea alba*, *Penicillium expansum*, and *Botrytis cinerea*. Both aqueous and ethanol extracts of garlic inhibited the growth of all fungi tested, especially Neofabraea alba, which is responsible for apple bull's eye rot [25].

In an intriguing study, Burian et al. reported that Sporothrix schenckii, a fungus that causes subcutaneous mycosis in Latin America, was treated in vivo with garlic using mice. The resultsshowed that garlic has effective antifungal properties against Sporothrix schenckii, and oral administration of garlic influenced the immune system of mice to respond more effectively to the infection by influencing the release of macrophage cytokines [26].

A. niger, A. flavus, F. solani, F. avenaceum, P. digitatum, R. stolonifer, and yeast (of the genus

Saccharomyces species) have all been linked to spoiled fruits in the research region.

This suggests that these fungi may be to blame for the fruit spoiling. This discovery is consistent with earlier studies by Baiyewu et al. and Chukwuka et al. [27] that described the isolation of A. niger, F. avenaceum, R. stolonifer and Nigerian pawpaw yeast.

Fresh fruit has lately been found to be a substantial source of chemical toxins, human and plantinfections, and other contaminants that could be dangerous to human health globally.

As a result of poor processing techniques, people also run the danger of contracting harmful fungus from fruits and vegetables because they are likely to be consumed raw by scavenging animals, particularly ruminants.

More specifically, fresh fruit poses a risk to food safety, and processing methods that are microbiologically deadly could result in issues.

Fresh fruit can be damaged by improper handling, which makes their products more likely to spoil or support pathogenic germs [27].

The investigation's findings ultimately lead to the conclusion that natural extracts of ordinary garlic can prevent the formation of fungus which causes post-harvest spoilage of fruits.

The growth of pathogens isolated on fruits was found to be inhibited by putative antifungal compounds identified in garlic bulb extracts. The findings of this study could be a crucial firststep in the development of plant biopesticides for the control of fruit rots.

V. CONCLUSION

This research was conducted to analyse that there are fungi that causes of fruits. These fungi were isolated and identified. The identified fungi were *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus flavus*. The efficiency of natural antifungal containing components like tulsi leaves, neem leaves, garlic bulb and turmeric bulb were used.

Garlic bulb were found to potential antifungal compound that inhibit the growth of pathogens.Garlic bulb were most activite to control and inhibit the growth of *Aspergillus flavus* among the various isolated fungi.
So, to conclude various formulation can done using garlic to reduce and control the post-harvestspoilage of various fruits. Garlic is natural, cost effective and easily available in the market which make its very desirable for any kind of formulation to fight against any fungal infections.

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