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# Identification of Fungal Species Associated with Contaminants and Pathogenicity on *Tamarindus indica* Fruits from Maiduguri Monday Market, Borno State Nigeria

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**Abstract:** A study of *Tamarindus indica* fruits rot was carried out in Maiduguri Monday Market located in The North- Eastern Nigeria. *Tamarind indica* fruits are showing sign of spoilage and fresh one were collected to ascertain the presence of contaminant and pathogenicity test was carry out to confirm further the fungal pathogen associated with fruits rot. We assessed the effects of temperature on the growth of colony diameter of the isolate. *In vitro* radial growth of each species of the fungal isolates (*Aspergillus niger*, *Rhizopus stolonifer*, *Ulocladium chartarum*, *Penicillium chrysogenum* and *Penicillium citrinum*) was measured at 37°C, 42°C, 47°C, 52°C, and 57°C for three weeks. Optimal growth for all the five-species occurred at 37°C, with slower growth at 47°C and 52°C. At 57°C, values of colony diameter reduced significantly for all the fungal isolates observed, however, there was a close relationship in values of colony diameter obtained for all the fungal species at 57°C. After three weeks, fungal colonies were digitally photomicrographed and colony opacity was assessed.

**Keywords:** *Tamarindus indica*, Sterilized and Unsterilized, Colony and Fungi

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## 1. Introduction

In Nigeria, many rural communities depend on plant species that are outside the conventional of agricultural research and development. These plant species are often referred to as underutilized crops and play a crucial role in the socioeconomic, food security, and food culture of the rural poor. *Tamarindus indica* L. widely known as Tamarind is widely grown as a subsistence crop for meeting local demands [3]. Numerous national programs have recognized tamarind as an underutilized crop with wider potential, since demand for products is substantial and the species can be incorporated into agroforestry systems [3]. Further exploitation of tamarind can therefore provide added incomes for poor rural people thereby improving their well-being [3].

*Tamarindus indica* L., is a multipurpose tropical fruits tree used primarily for its fruits, which are eaten fresh or processed, used as a seasoning or spice, or the fruits and seeds are processed for non-food uses [3]. Tamarind belongs to the

dicotyledonous family Leguminosae which is the third largest family of flowering plants with a total of 727 genera recognized and the number of species is estimated at 19,327 [6]. The fruit is an indehiscent subcylindrical pod with fibrous pulp containing seeds 10 x 18 x 4 cm long and approximately 2 cm wide, straight or curved, velvety, rusty brown; the shell of the pod is brittle [5]. Tamarind trees have positive environmental benefits as they provide perennial cover protecting the soil and act as effective windbreaks [4]. Tamarinds are also found growing well in the semi-arid region of northern Nigeria. Gunasena [4] reported that tamarind grown especially in arid climatic regions, can provide, store and recycle plant nutrients, and give stability to the soil. Several studies have demonstrated the antimicrobial potential of leaves and fruits of tamarind [8, 9].

The objective of the research piece therefore is to establish the pathogenicity and contaminants associated with *Tamarindus indica* fruits and to ascertain the temperature optimum for its storage with the aim of ensuring its use by the local community.

## 2. Materials and Methods

### 2.1. Sample Collection

Samples of *Tamarindus indica* fruit (fresh and the old) were purchased randomly from the local marketers in Maiduguri Monday market, Borno State, Nigeria. These samples were brought to the laboratory in polythene bags for study.

### 2.2. Isolation and Culturing

The medium employed during the experiment was Potato Dextrose Agar (PDA). *Tamarindus indica* fruits showing signs of rot and clean ones were obtained and cut into small pieces with sterilized scalpel. The surface sterilized pieces were transferred to the solidified PDA plates. The pieces were placed at an approximately equal distance from each other and then incubated at room temperature for 3-4 days. Some unsterilized pieces were plated in the same manner to serve as unsterilized treatments. The type of experiment design used was the Complete Randomized Design; each treatment was replicated four (4) times. Both the pouring of medium and planting of cut pieces of fruits were done under the aseptic conditions to minimax the chance of contamination. Fungal growth was measured after the fourth day of incubation.

The reverse side of the colonies in PDA plates was measured in millimeter with a ruler. Slides were prepared from each isolated colony of the fungi by placing a small amount of

the fungal material in a drop of water on clean slides and a drop of Lugol's iodine solution.

The mixture was then spread thinly with two fine needles, covered with a coverslip and observed under the Nikon microscope for detailed structures. Identification was based on morphological characteristics of the fruity body, the colour of the mycelium on the reverse side of the PDA plate and growth characteristic of the colony as well as vegetative and reproductive structure. This identification was further confirmed by the descriptions given by Samson and Reenen-Hoekstra [10].

## 3. Results

The result of the fungal species isolated from the *Tamarindus indica* fruit (surface sterilized and unsterilized) shows the presence of *Aspergillus niger*, *Rhizopus stolonifer*, *Ulocladium chartarum*, *Penicillium chrysogenum* and *Penicillium citrinum*.

Table 1 shows the isolated fungal species from surface sterilized and unsterilized fruits and their respective characteristics. The species of fungi associated with the surface sterilized treatment were; *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium chrysogenum* and *Penicillium citrinum* while for the surface unsterilized *Ulocladium chartarum*, *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium chrysogenum* and *Penicillium citrinum* were isolated.

**Table 1.** Fungal Isolates from fruits (surface sterilized and unsterilized) and their Characteristics patterns of spoilage.

Sample	Isolated fungal species	Characteristics pattern of spoilage
<i>Tamarindus indica</i> (Surface Sterilized)	<i>Aspergillus niger</i>	Dark-brown, light brown and release of water substance.
	<i>Rhizopus stolonifer</i>	The dark-brown release of water substance.
	<i>Penicillium chrysogenum</i>	Light green and release of water substance.
	<i>Penicillium citrinum</i>	Dark green, light green and release of water substance.
	<i>Ulocladium chartarum</i>	Dark-brown and release of water.
	<i>Aspergillus niger</i>	Dark-brown light brown and release of water substance.
<i>Tamarindus indica</i> (Surface unsterilized)	<i>Rhizopus stolonifer</i>	Dark brown and release of water substance.
	<i>Penicillium chrysogenum</i>	Light and release of water substance.
	<i>Penicillium citrinum</i>	Dark-green, light green and release of water substance.

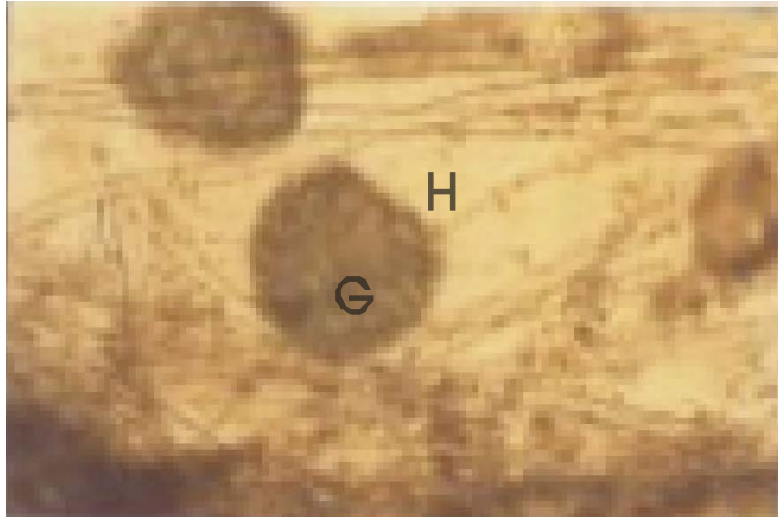
**Table 2.** Pathogenicity test.

Nature of the fruits inoculated	Fungal species isolated	Spoilage pattern produced
<i>Tamarindus indica</i> (Surface sterilized)	<i>Aspergillus niger</i>	Dark and light brown and release of water substance.
	<i>Rhizopus stolonifer</i>	Dark-brown release of water substance
	<i>Penicillium chrysogenum</i>	Light green and release of water substance.
	<i>Penicillium citrinum</i>	Dark and light green, the release of water substance.
<i>Tamarindus indica</i> (Surface unsterilized)	<i>Ulocladium chartarum</i>	Dark-brown and release of water.
	<i>Aspergillus niger</i>	Dark-brown light brown and release of water substance.
	<i>Rhizopus stolonifer</i>	Dark brown and release of water substance.
	<i>Penicillium chrysogenum</i>	Light and release of water substance.
	<i>Penicillium citrinum</i>	Dark-green, light green and release of water substance.

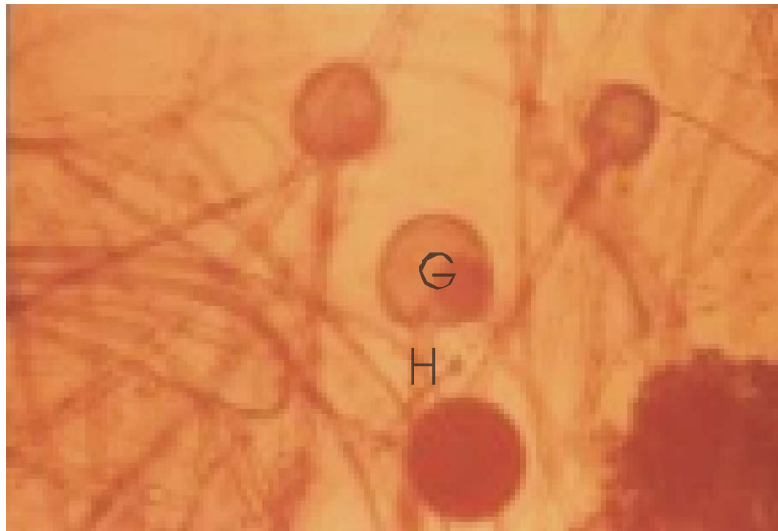
Photomicrographs of the fungal species are shown in figure 1-5 at a magnification of X40. Figure 1 reveals the photomicrograph of *Aspergillus niger* showing the spore (S), the sporangium (G) and the hyphae (H). Figure 2 shows the

photomicrograph of *Rhizopus stolonifer*. Figure 3 shows the photomicrograph of *Penicillium chrysogenum*.

Figure 4 shows the 47°C, 52°C, of *Penicillium citrinum* and figure 5 shows the photomicrograph of *Ulocladium chartarum*.



**Figure 1.** *Aspergillus niger* at  $\times 40$ . H= hyphae and G=Sporangium.



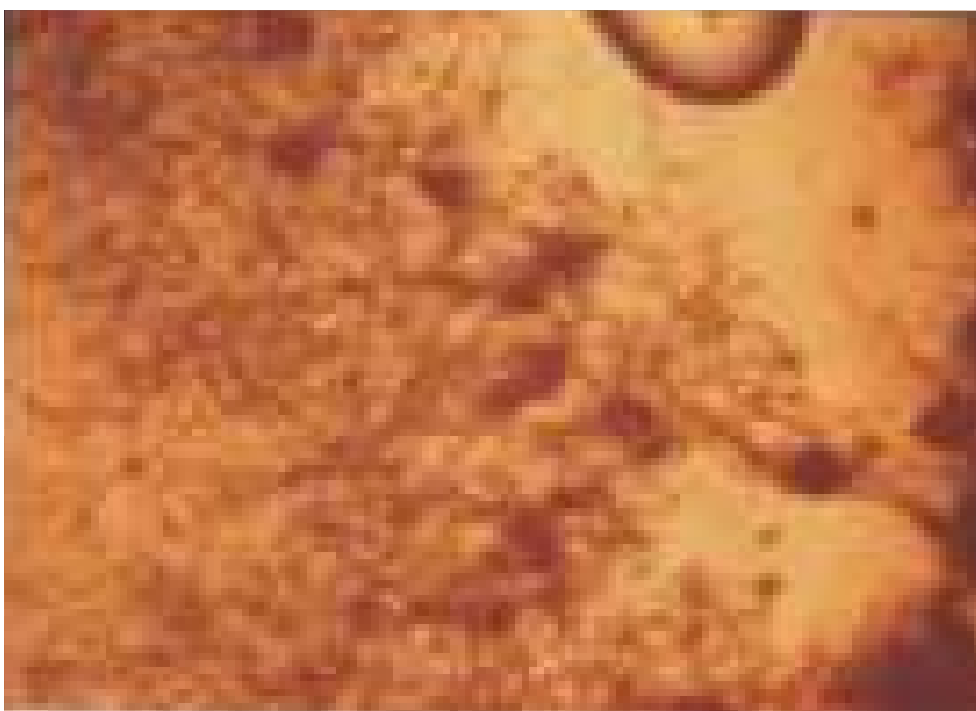
**Figure 2.** *Rhizopus stolonifer* at  $\times 40$  Where H=hyphae G=sporangium.



**Figure 3.** *Penicillium chrysogenum* at  $\times 40$ .



**Figure 4.** *Penicillium citrinum* at  $\times 40$ .



**Figure 5.** *Ulocladium chartarum* at  $\times 40$ .

Figure 6 shows the effect of temperature on colony diameter of the various fungi isolated from the fruit of *Tamarindus indica*. The increase in temperature caused a significant reduction in colony diameter for all the fungal species. At 37°C, most fungal isolates showed little or no reduction in colony diameter. Values of colony diameter recorded for *A. niger* at 37°C was higher than those obtained for *R. stolonifer*, *P. chrysogenum* *P. citrium* and *U. chartarum* while at 42°C, values obtained for *U. chartarum* was noted to be higher than for all other isolates. The values

of colony diameter recorded for *A. niger* and *U. chartarum* were significantly higher than the values recorded for the other fungal isolates while the values obtained for *R. stolonifer*, *P. chrysogenum* *P. citrium* showed no significant difference in values of colony diameter. The same trend was observed at temperatures of 47°C and 52°C respectively. At 57°C, values of colony diameter reduced significantly for all the fungal isolates observed, however, there was a close relationship in values of colony diameter obtained for all the fungal species at 57°C.

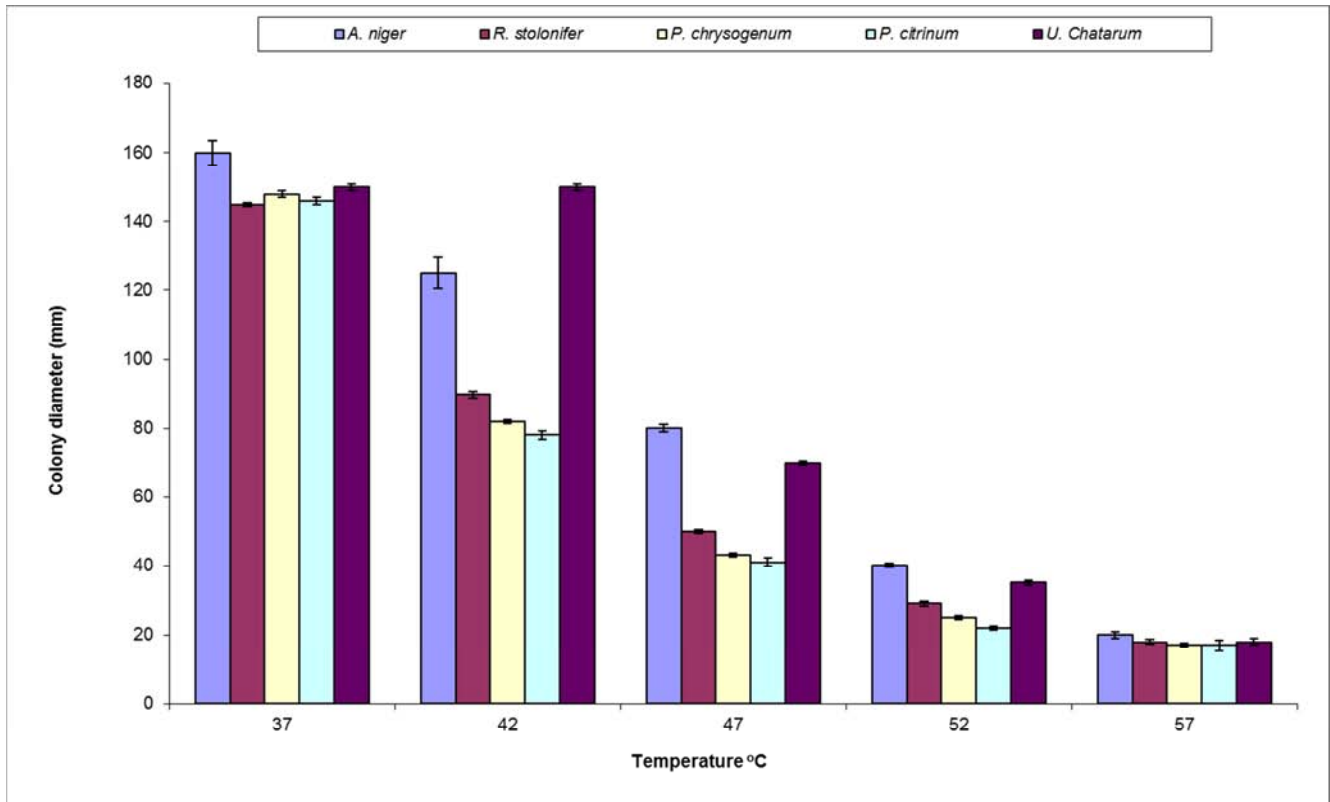


Figure 6. Effect of Temperature on Colony Diameter of Fungal isolates.

## 4. Discussion

Fungal contamination associated with *Tamarindus indica* fruits in Maiduguri Monday market has been established. *Aspergillus niger*, *Rhizopus stolonifer*, *Ulocladium chartarum*, *Penicillium chrysogenum* and *Penicillium citrinum* are pathogens found associated with the spoilage of *Tamarindus indica* fruit. This was further confirmed by pathogenicity test. Lokesha and Shetty [7] reported that stony fruits disease caused by the fungal pathogen *Pestalotia macrotricha* Syd in *Tamarindus indica* fruits makes the fruits hard and stony with fibrous structures. Sap rot and white rot, which might be caused by several diverse fungi, are the major diseases of *Tamarindus indica* fruits [3]. The diseased fruits caused by fungal pathogens and contamination changes the colour, appearance and triggers the release of water substances. These led to the deterioration and decreased the shelf life of the fruits. Deterioration of fresh commodities can result from physiological breakdown due to natural ripening processes, water loss, temperature injury, physical damage, or invasion by microorganisms [11].

The prevalence of fungi as the spoilage organism of fruits and vegetables is due to a wide range of factors which are encountered at each stage of handling. From pre-harvest to consumption and is related to the physiological and physical conditions of the produce as well as the extrinsic parameters to which they are subjected [2]. The pre-harvest handling technique of the fruits, optimal temperature condition and poor storage facilities are likely factors that determine

infestation and degree of pathogens. These factors can interact at different stages of the postharvest-storage handling and influence by environmental conditions.

Colonization by the fungal pathogen ascertains the level of impact of contamination and the total spoilage of the fruits. The colonization process involves the ability of the microorganism to establish itself within the produce and this is initiated when the microorganism (following adhesion and release of enzyme) degrade certain specific cell wall polymers such as protopectin, the cementing substance of the produce [1]. Variation regarding the invading fungal pathogen and colonization occurred and were more susceptible to the unsterilized surface treatment of the fruits than the surface sterilized fruits. This may be due to the presence of inducing factors that make it prone to fungal pathogens. Effiuvwe were [2] reported that high moisture and relative humidity led to greater fungal growth in agricultural produce and thus low storability of fruits and vegetables.

The underutilized fruits *Tamarindus indica* is valued mostly for its fruits, which can be used for many purposes. The unique sweet/sour flavor of the fruits is popular for cooking, beverages and flavouring. It has a high concentration of vitamin C, which may affect the dietary and nutritional content of rural people.

This nutritional value of the fruits can further be utilized in processing and packaging into varieties of food product for local consumption and in turn increase the livelihood of the local people.

To achieve sustainable development in agriculture, food

national security and increased in the livelihood of the rural people; the need for seedling plantation of this fruits are important and to consider all the resources and techniques involved in proper pre-harvest and postharvest handling circle of this fruits.

## 5. Conclusion

Fungal pathogens that were isolated and identified in this study revealed to be the potential contaminants of *Tamarindus indica* fruit in Maiduguri Monday market. These pathogens can cause ill health when ingested by humans. However, the stage at which these pathogenic fungi get into the fruits has not been established. It is possible that the identified fungal pathogens are residing right on the farm or during postharvest handling. It is of importance to investigate and evaluate the point of contact of those fungal contaminants to apply proper biological control that is sustainable. The increase in temperature has been established to affect colony diameter while 37°C is the optimal growth temperature that was ascertained for all fungal colony identified. Therefore, the temperature is going to be an important factor in designing optimal conditions during postharvest storage of this fruits.

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