Isolation And Identification of Fungi Associated with Dried Fruits of Ziziphus Spina-Christi (L.) Willd, In Dutse Jigawa State, Nigeria

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ABSTRACT

This research was carried out on the isolation and identification of fungi associated with the dried fruits of Ziziphus spina-christi (L.) Willd (Christ's thorn). Sample fruits were collected from ten (10) mother trees in Dutse, and air dried for eight (8) weeks. The Pulps were extracted from the fruits with the aid of pestle and mortar; one (1g) of the pulp was placed in a beaker containing nine of (9mls) distilled water. The sample solution was serially diluted in this manner 10^1 10^2 ---10^6 in order to reduce fungal load in the sample. A drop of the serially diluted solution was placed on the prepared potato dextrose agar media under sterilized environment and incubated at (37^0C) for 48 hours. The pure/axemic culture were obtained by several transfer of colony growth from PDA plates aseptically i.e. (Sub-culturing until axemic culture were obtained). Identification was done macroscopically and microscopically. Result shows that Rhizopus spp are found to have the highest percentage 8.2 (64%) of occurrence which makes it the most dominant while Aspergillus Spp, have 3.2 (25%) percentage of occurrence thus Fusarium spp had 1.4 (11%) percentage of occurrence. Therefore., it is concluded that Aspergillus, Fusarium, and Rhizopus are associated with the dried fruits of Ziziphus spina-christi (Christ's thorn). Aspergillus species are associated with food spoilage and can significantly impede the quality and safety of the fruits. Therefore, improved Post-Harvest Handling in form of better drying and storage practices, routine fungal contamination assessments using molecular and microscopic techniques, more so, further research should be encouraged on inhibiting mycotoxin-production capabilities of isolated fungi.

Keywords: *Ziziphus spina-christi, Dried fruit, Isolation, Identification, Nutritional value and Fungi.*

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INTRODUCTION

Ziziphus spina-christi is widely cultivated for its edible fruits across the Sahara, Tunisia, Algeria, Niger, Nigeria, East Africa, Israel, and Iran (Dhanalekshmi *et al.,* 2022). The fruit is eaten fresh, dried, or processed into flour, paste, or cakes, although its flavor and texture are inferior to other *Ziziphus species* (Hassan *et al.,* 2025). Traditionally, the plant is used as an emollient, astringent, and to treat various ailments, including abscesses, boils, eye

inflammation, fever, and toothache. In some regions, it's used as an abortifacient and rodent pest control (Bussmann *et al.,* 2025). The fruit flesh of *Z. spina-Christi* is rich in carbohydrates, including starch (21.8%), sucrose (21.8%), glucose (9.6%), and fructose (Abdulrahman *et al.,* 2022). It is a good source of carotene, retinol, and ascorbic acid. Per 100g dry matter, the fruit contains 9.3% water, 4.8% protein, 0.9% fat, 80.6% carbohydrates, 4.4% ash, and has an energy value of 314 kcal (Angami, 2020). The fruit is also a rich source of minerals and vitamins, including calcium (140mg), iron (3mg), thiamine (0.04mg), riboflavin (0.13mg), niacin (3.7mg), and ascorbic acid (30mg) (Angami, 2020). Other studies have reported similar values, with Salih and Yahia (2015) noting 10.53% water, 2.55% fat, 4.34% protein, and 74.31% carbohydrates, with mineral contents including calcium (173mg), potassium (840mg), and iron (1.1mg) per 100g DW. These findings highlight the nutritional value of *Z. spina-Christi* fruit, making it a valuable resource for food and medicine (Abdulrahman *et al.,* 2022). Fruits are harvested green, stored for 6 days (room temperature) or 22 days (10°C), and eaten fresh, dried, or processed (Jayaraman and Gupta, 2020). Rich in carbs (starch, sucrose, glucose, fructose), they're a local market commodity. The tree aggressively colonizes, forming impenetrable thickets, but its dry fruits are prone to fungal contamination (Bunyard, 2024). *Ziziphus spina-Christi* fruits (Jujubes) are a valuable food source with health benefits, preserved through drying. They contain 14% sugar and 1.6% vitamin C (Hussein., 2019)

Fungal pathogens pose a significant threat to the post-harvest integrity of *Z. spina-Christi* dry fruits, causing decay, spoilage, and loss of nutritional value (Zohair *et al.,* 2025). Additionally, certain fungal species may produce mycotoxins, compounds harmful to human and animal health, further underscoring the importance of understanding the fungal community associated with these fruits (Adeniyi *et al.,* 2024). Fungal contamination of dried *Ziziphus spina-christi* fruits poses significant public health risks due to the potential for mycotoxin production. Mycotoxins such as aflatoxins, ochratoxins, and fumonisins are potent carcinogens and can cause acute and chronic health effects (Ostry *et al*., 2017). Stringent quality control measures, including proper drying, storage conditions, and regular screening for fungal contamination, are essential to ensure food safety (Fathi, *et al.,* 2022). In mitigating fungal contamination, various preservation techniques and antifungal strategies have been explored. Physical methods such as controlled atmosphere storage, irradiation, and the use of desiccants can inhibit fungal growth by altering environmental conditions (Srivastava *et al.,* 2021). Chemical preservatives, including sorbic acid, benzoic acid, and sulfur dioxide, are commonly used in the food industry to control fungal contamination (Davidson and Branen, 2021). Regulatory agencies worldwide have established limits for mycotoxins in food products to protect consumer health. For instance, the European Union has set maximum levels for aflatoxins, ochratoxin, and patulin in various food commodities. The United States Food and Drug Administration (FDA) and the Food and Agriculture Organization (FAO) also provide guidelines for mycotoxin limits and testing protocols (FAO, 2020). Compliance with these regulations requires regular monitoring and testing of dried fruits for mycotoxin contamination. Analytical methods such as Enzyme-Linked Immunosorbent Assay (ELISA), high-performance liquid chromatography (HPLC), and liquid chromatography-tandem mass spectrometry (LC-MS/MS) are commonly used for mycotoxin detection and quantification (Janik *et al.,* 2021). Despite the ecological and economic importance of *Z. spina-Christi* dry fruits, comprehensive studies on the fungal communities inhabiting them are scarce. Therefore, this research aims to fill this knowledge gap by systematically isolating and identifying the fungal species associated with *Z. spina-Christi* dry fruits in view of revealing the fruit quality, safety, and post-harvest management practices.

MATERIALS AND METHODS

Description of study site

The study was carried out at Forestry Nursery of the Department of Forestry and Wildlife Management, Federal University Dutse, Jigawa State. It lies between latitude 11.00°N to 13.00°N and longitudes 8.00°E to 10.15°E (Gidado *et al.,* 2023). (Figure 1) The study area Dutse covered by Sudan area and also characterized by hot wet summer and cool dry winter with average raining season of 3-5 (644 m) (Olatunde *et al.,* 2026; Jibo *et al.,* 2021). Sunshine hours indicates that the town enjoys 10-11 hours of sunshine depending on the season. The topography is characterized by high land area which is almost 750m. Soil tends to be fertile ranging from sandy-loam (Salami *et al.,* 2024; Bidemi *et al.,* 2026).).

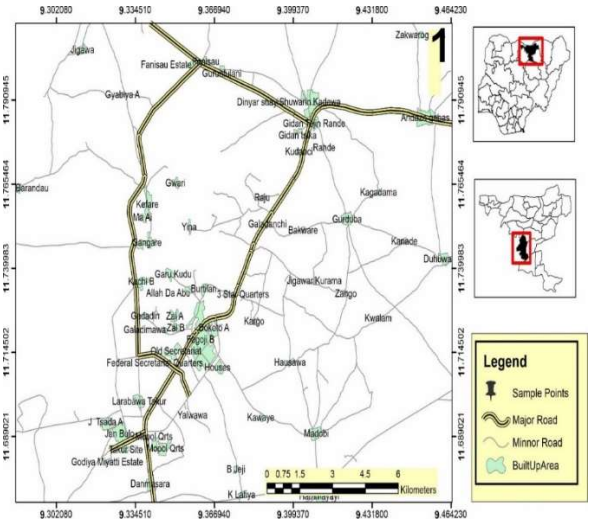

Figure 1: Map of Dutse Local Government**; Source:** Salami *et al*., 2020.

Table 1: Macroscopic and Microscopic features of identified fungi.

S/N	Fungi identified	Macroscopic features	Microscopic features
1	*Rhizopus spp*	White fluffy mycelium mat with black sporangia. *Rhizopus species* are commonly forms in various food products, including fruits where they can cause soft rot.	Broad and ribbon like, typically 6-15 micrometer in diameter with slightly oval shape containing numerous sporangiospores. It has smooth to slightly roughened surface.
2	*Aspergillus spp*	Colonies are typically powdery or cottony containing black, yellow to green colonies.	Smooth walled or slightly roughened surface. It has erected unbranched structures arising from foot cells with spherical shape the ranges from 20-30 micrometer in diameter.
3	*Fussarium spp*	Colonies are typically woolly, cottony, dense and leathery with white to brown color.	Smooth walled or slightly branching surface. It has treelike shape; it sizes ranges from 20-50 micrometer in diameter

The mean annual temperature in the area supports the growth of variety of plants. The area's relatively uniform climate and soil conditions ensured that the results obtained from the study were reliable and applicable to similar agro-ecological zones. The topography of the area is generally flat, with a high land area of approximately 750 meters. The soil type in this region is predominantly sandy-loam, as reported in previous studies (Ilu et *al.*, 2023; Garba *et al*., 2021). This soil type is characterized by a mix of sand, silt, and clay, providing a suitable medium for plant growth

Sample collection

Sample fruits of Christ's thorn (*Ziziphus spina-christi*), was collected from ten (10) mother trees in Dutse, in Jigawa state. The samples were air dried for eight (8) weeks.

Sample Preparation

The pulp was extracted with the help of pestle and mortar; a small portion of the pulp was placed in a beaker containing distilled water. The sample solution was serially diluted in this manner 10^1 10^2 …10^6 in order to reduce fungal load in the sample.

Preparation of Culture Media

Potato Dextrose Agar (P.D.A) was used for culturing fungi which is prepared according to the manufacturers' instruction and autoclave at 121^0 C for 15 minute.

Culturing of Fungi

The prepared culture media was sterilized with the help of autoclave to ensure that the media are free of contamination. A drop of the serially diluted solution was placed on the prepared potato dextrose agar media under sterilized environment and incubated at (37^0C) for 48 hours.

Isolation of fungi

The isolation technique employed by Chiejina (2008) was adopted in the present study. A drop of the preferred solution was placed on the agar media (PDA) and incubated at 37^0C room temperature for three (3) days. The pure axemic culture was obtained by several transfer of colony growth from PDA plates aseptically i.e. (Sub-culturing until axemic culture were obtained).

Identification of fungi

Identification was done macroscopically and microscopically. For macroscopic identification, colony characteristics such as appearance change in medium colour and growth rate was observed on the petri plates. (Table 1) For microscopic identification, a thin smear of fungi isolates from 5–7-day old cultures were inoculated aseptically on a clean glass slide using a sterile inoculating loop. A drop of lactophenol cotton blue was added and the mixture was covered with a cover slip and viewed under X40 objective lens of the light microscope. Shapes of the conidia and conidiophores were taken note of. These features were matched with standards described (Deshmukh *et al.,* 2020) as reported by Liamngee *et al.,* (2019)

Fungal spore count procedure

Table 2: Fungal Spore Counts

S/N	PDA	Spore cells counted
1	10^2	100,000 spores/ml
2	10^4	90,000 spores/ml
3	10^6	70,000 spores/ml

Fungal spores were counted using hemocytometer counting chamber. hemocytometer is a precision instrument used to count cells or spores in a defined volume. (Table 2) Fungal spores were collected from the cultured media using sterile techniques, suspend the spores in a known volume of diluent to create a homogenous spore solution, it was mix thoroughly to

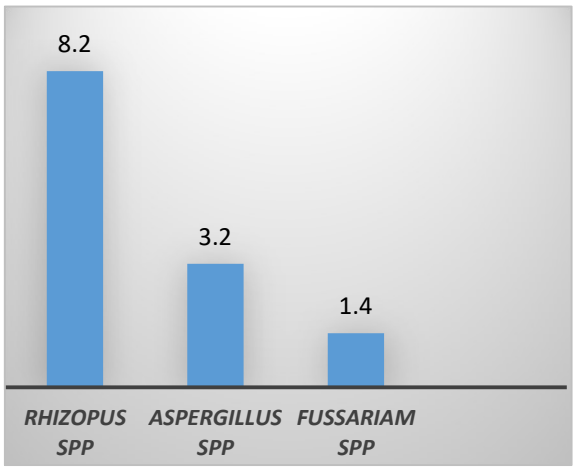

Figure 2: Fungal Spores identified in Dry Fruit of *Zizipus Spina Christi*

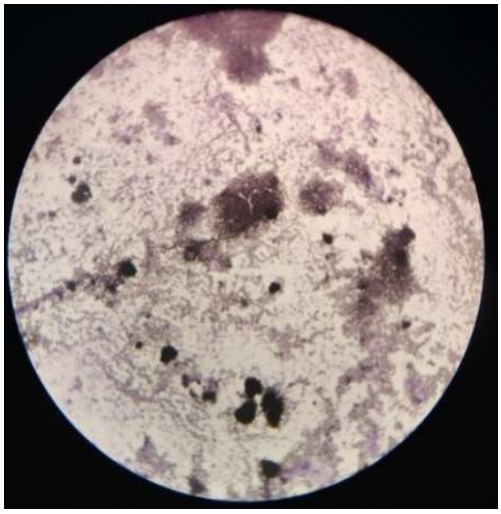

Plate 1: Physical appearance of *Rhizopus Spp* under microscope

ensure even distribution, coverslip was place on the hemocytometer, hemocytometer was loaded with the volume of about 10-20 microliter. Capillary action draws the suspension into the chamber. The hemocytometer was placed under a microscope; the focus was on the grid using a lower-power objective (10x or 40x). The grid consists of 9 large squares; each is subdivided into smaller squares. 4-5 large squares were selected to count spores within. Count only those spores that are fully inside the square or touching the top and left boundary lines those on the bottom and right lines were exclude to avoid double counting. The same procedure was use for each dish.

Data analysis

Data were analyse using descriptive statistics such chart and table were also used to indicates the frequency of occurrence.

RESULTS AND DISCUSSION

RESULTS

The above table indicates the total number of spores spotted in each Potato Dextrose Agar (PDA) cultured media and the spores were counted with the aid of hemocytometer under microscope. Figure 2 revealed the percentage of fungi associated with Dry fruit of *Zizipus Spina-Chrisit*, Result shows that *Rhizopus spp* are found to have the highest percentage (64%) of occurrence which makes it the most dominant while *Aspergillus Spp*, have (25%) percentage of occurrence thus *Fusarium spp* had (11%) percentage of occurrence.

DISCUSSION

The study revealed the presence of three primary fungal General *Rhizopus, Aspergillus*, and *Fusarium* isolated from dried *Ziziphus spina-christi* fruits. These fungi are well-known for their ability to thrive in nutrient-rich, low-moisture environments typical of dried fruits, making them significant agents of spoilage and potential producers of harmful metabolites. *(Plate 1) Rhizopus spp*., particularly *Rhizopus stolonifer*, was identified as common contaminants in the dried fruits. This genus is associated with soft rot due to its capacity to secrete hydrolytic enzymes that break down fruit tissues. The presence of *Rhizopus* suggests suboptimal drying practices or exposure to high humidity during storage (Elshafie *et al.,* 2022). Gidado *et al.* (2025), reported similar results from *Detarium microcarpum* which showed that *Rhizopus stolonifer, Apergillus niger Fusarium oxysporum, Fusarium moniliforme* were found to be associated with the dry fruits with frequencies of occurrence of 42%, 32%, 10% and 16% respectively. Similarly, a study on tomato fruits revealed that Aspergillus niger and Yeast were the most virulent isolates, causing the most severe rot at both Na'ibawa market (29.63% each) and Wudil market (34.48% and 31.03%, respectively). Rhizophus stolonifer showed moderate virulence, while Aspergillus flavus and Fusarium spp. exhibited lower pathogenicity. The similarity in fungal pathogens suggests that Ziziphus spina-christi and tomato fruits may share common fungal spoilage profiles, highlighting the potential for shared management strategies to mitigate post-harvest losses (Jahun *et al.,* 2021). Although *Rhizopus* is not known to produce mycotoxins, its rapid colonization can compromise the physical and sensory quality of dried fruits, leading to economic losses (Seethapathy, 2025). (Plate 2) The genus *Aspergillus* was the most frequently isolated, aligning with its known xerophilic nature, which allows it to thrive in low water activity environments like dried fruits.

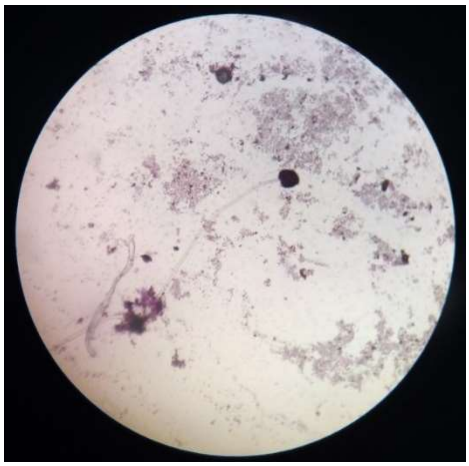

Plate 2: Physical appearance of *Aspergillus Spp* under microscope.

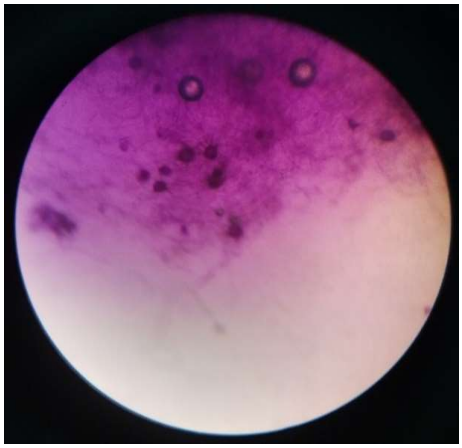

Plate 3: Physical appearance of *Fussarium Spp* under microscope.

Aspergillus spp. was prominent among isolates and is significant due to its ability to produce aflatoxins, which are highly carcinogenic and regulated in food products worldwide (Mamo, *et al.,* 2021). The presence of *Aspergillus spp*. indicates poor storage practices or exposure to contaminated environments. These fungi emphasize the need for stringent monitoring of mycotoxin levels in dried fruits to ensure consumer safety. (Plate 3) Although less frequently isolated, *Fusarium spp* was detected in the dried fruits. Species such as *Fusarium solani* and *Fusarium oxysporum* are notable due to their ability to produce mycotoxins like fumonisins and trichothecenes, which pose serious health risks to humans (Shabeer *et al*., 2021). The presence of *Fusarium* may indicate contamination during harvesting, as these fungi are commonly soilborne and can be introduced to fruits through handling or improper sanitation. *Fusarium* contamination in dried fruits is concerning, as mycotoxins are stable and can persist even in low-moisture environments (Ezekiel *et al.,* 2020).

Conclusion

It was concluded that diverse range of fungi were identified on the dry fruits of *Ziziphus spina-christi*, including genera such as *Aspergillus, Fusarium*, and *Rhizopus*. These fungi are commonly associated with food spoilage and can significantly impact the quality and safety of the fruits. *Aspergillus* species were known for its potential for mycotoxin production, such as aflatoxins, which are harmful to human health. Environmental conditions such as humidity, temperature, and inadequate post-harvest handling were likely contributors to fungal contamination, as these factors provide favorable conditions for fungal growth. Proper identification of fungal species provides a foundation for understanding contamination patterns and implementing targeted control measures.

Recommendations

Improved Post-Harvest Handling implement better drying techniques and storage practices to reduce moisture content, which is a key factor in fungal growth. Use sealed and airtight packaging to limit exposure to environmental spores. Storage Conditions maintain low humidity and cool temperatures during storage to inhibit fungal growth. Regularly monitor stored fruits for signs of contamination. Fungal Monitoring and Control which include routine fungal contamination assessments using molecular and microscopic techniques. Consider antifungal treatments or natural preservatives, such as plant extracts, to limit fungal growth. Further Research to investigate the potential mycotoxin-producing capabilities of isolated fungi. Study the effectiveness of natural or synthetic antifungal agents to control contamination. Explore breeding or genetic modifications of *Ziziphus spina-christi* varieties to enhance resistance to fungal colonization. Public Awareness Campaigns educate farmers, producers, and consumers about fungal contamination risks and preventive measures to ensure food safety and reduce post-harvest losses.

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