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Seasonal Variation in Fungal Pathogens Affecting Sweet Pepper (*Capsicum annuum* L.) in the Agricultural Zones of Sokoto State, Nigeria

[1]Abubakar Shehu, [1]Sanusi Muhammad, [1]Habsatu Shehu, [2]Abubakar Muhammad Nasiru and [3]Abdulkadir Shehu

[1]Department of Plants Sciences, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria
[2]Department of Forestry and Environment, Faculty of Agriculture, Usmanu Danfodiyo University, Sokoto, Nigeria
[3]Department of Biological Sciences, Faculty of Science, Sokoto State University, Sokoto, Nigeria

ABSTRACT

Background and Objective: Diseases significantly constrain sweet pepper (*Capsicum annuum* L.) production in Sub-Saharan Africa, including Nigeria, with phytopathogenic fungi causing substantial yield losses. This study examined the seasonal variation in fungal pathogens affecting sweet peppers in Sokoto State, Nigeria. **Materials and Methods:** Fungal isolates were obtained from visibly damaged sweet pepper samples collected across the four agricultural zones of the state during the dry season. Potato Dextrose Agar (PDA) medium was used for isolation and identification was performed using a DA2-180M Halogen Lamp Microscope (6V, 20W). **Results:** Eight fungal species were identified: *Cephalosporium acremonium*, *Mucor hiemalis*, *Rhizopus oryzae*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifer* and *Candida albicans*. Morphological traits aligned with established taxonomic descriptions, with *Aspergillus niger* and *Aspergillus fumigatus* identified as potential pathogens in both dry and rainy seasons. **Conclusion:** The study highlights the diversity of fungal pathogens affecting sweet peppers and recommends further research on their pathogenic potential, molecular characterization and environmental monitoring.

KEYWORDS

Fungal pathogens, sweet pepper, Sokoto State, Nigeria, isolation, identification, *Aspergillus*, *Mucor*, *Rhizopus*, *Candida*

INTRODUCTION

The pepper grown worldwide consists of approximately 22 wild species and five domesticated species. The five domesticated species include *C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum* and *C. pubescens*[1]. *Capsicum* species can be categorized based on fruit or pod characteristics such as pungency, color, shape, intended use, flavor and size. Despite these diverse traits, most commercially cultivated pepper varieties globally belong to *C. annuum*[2]. There are an estimated 1,600 varieties of pepper (*Capsicum annuum*) worldwide[3].

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World pepper production has experienced significant growth over the past two decades, with dry types increasing from 2 to about 4.5 million tons and fresh types growing from 17 to 36 million tons annually[4]. The cultivated area has expanded correspondingly, with a 35% increase in harvested hectares. Fresh pepper is now grown in 126 countries across all continents, with China leading as the largest producer (18 million ton annually), followed by Mexico (3.5 million ton annually)[5]. Peppers thrive in various climatic and environmental conditions and can be cultivated both in open fields and greenhouses, making them one of the most versatile crops[6]. The economic value of pepper production has risen significantly, with dry pepper valued at \$3.8 billion and fresh pepper at \$30.208 billion[7]. This increase is attributed to the rising demand for peppers in international trade, as well as their high antioxidant content and role in fruit pigmentation[8].

Diseases pose major challenges to sweet pepper production, especially in Sub-Saharan Africa, including Nigeria. Phytopathogenic fungi, bacteria and viruses are significant contributors to yield losses. Between 1999 and 2004, the National Programme for Agriculture and Food Security (NPAFS) reported a decline in pepper yields from 2,939 to 2,912 ton and from 2,744 to 2,469 ton[9]. Even with an increase in cultivated land from 22.5 to 26.92 ha between 2007 and 2009, the yield only marginally improved from 5,532 to 6,063 ton[10]. These declines are attributed to a combination of biotic and abiotic factors, such as weeds, pests, diseases and environmental stress[11,12]. Identifying specific diseases responsible for reduced yields in sweet peppers in Sokoto State is crucial for developing effective control strategies and improving farmer productivity. This study investigated the seasonal variation in fungal pathogens affecting sweet pepper (*Capsicum annuum* L.) across agricultural zones in Sokoto State, Nigeria and assessed their implications for crop health and management practices.

MATERIALS AND METHODS

Sources of isolates: The fungal isolates (*Cephalosporium acremonium*, *Mucor hiemalis*, *Rhizopus oryzae*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifer* and *Candida albicans*) used in this study were obtained from the samples of physically damaged sweet pepper (*Capsicum annuum* L.) collected from across the four Agricultural Zones of Sokoto State, Nigeria, in April, 2024 during the dry season. The samples (physically healthy and damaged), were each collected in a separate sterile polythene bag and transported to the mycology laboratory of the Department of Plants Science, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University, Sokoto (within 2 hrs of collection) for the isolation of fungi.

Isolation of fungal pathogens: Potato Dextrose Agar (PDA) medium was utilized for fungal isolation in this study, following the preparation guidelines provided by the manufacturer. Small portions of sweet pepper fruits were aseptically cut into 5 mm pieces using sterilized scissors and placed at the center of Petri dishes containing solidified PDA. The plates were incubated in the dark at room temperature (32±2°C) for 72 hrs. Fungal colonies emerging from the plates were subsequently transferred to fresh medium and sub-cultured repeatedly to obtain pure cultures.

Identification of fungal pathogens (phenotype): The Microscope used was DA2-180M with HALOGEN LAMP 6V 20W NO. 000059. In the Mycology Laboratory of the Plants Science Department, to examine the colony characteristics to establish the identity of fungi. A sterile needle was used in taking a small portion of the hyphae containing spores on the sterile glass slide, stained with lacto phenol cotton blue and then examined under the microscope for fungal structures. The morphological characteristics and appearance of the fungi isolated were confirmed and authenticated using the Illustrated Keys, Glossary and Guide to Literature Manual of Dugan[13]. The isolated fungus was identified based on its colony characteristics, including color and shape, as well as morphological features observed under the microscope. These characteristics were further confirmed and validated.

The identification of the isolated fungus was based on its colony and morphological traits, including color and shape observed under the microscope. These morphological features and the overall appearance of the fungi were verified and validated using the Mycological Atlas by Dugan[13].

RESULTS

The study highlights several fungal species, focusing on their characteristics and significance. *Candida albicans*, a part of the normal human microbiota, can act as an opportunistic pathogen and cause infections such as thrush. Among molds, *Rhizopus stolonifer* is notable for its rapid growth and production of black sporangia, often contributing to food spoilage. Similarly, *Rhizopus oryzae* is associated with both food spoilage and fermentation processes. *Mucor hiemalis*, a zygomycete mold, is commonly found in soil and decaying organic material, recognizable by its white to greyish appearance. The genus *Aspergillus* includes several species of interest: *Aspergillus flavus* produces carcinogenic aflatoxins, affecting

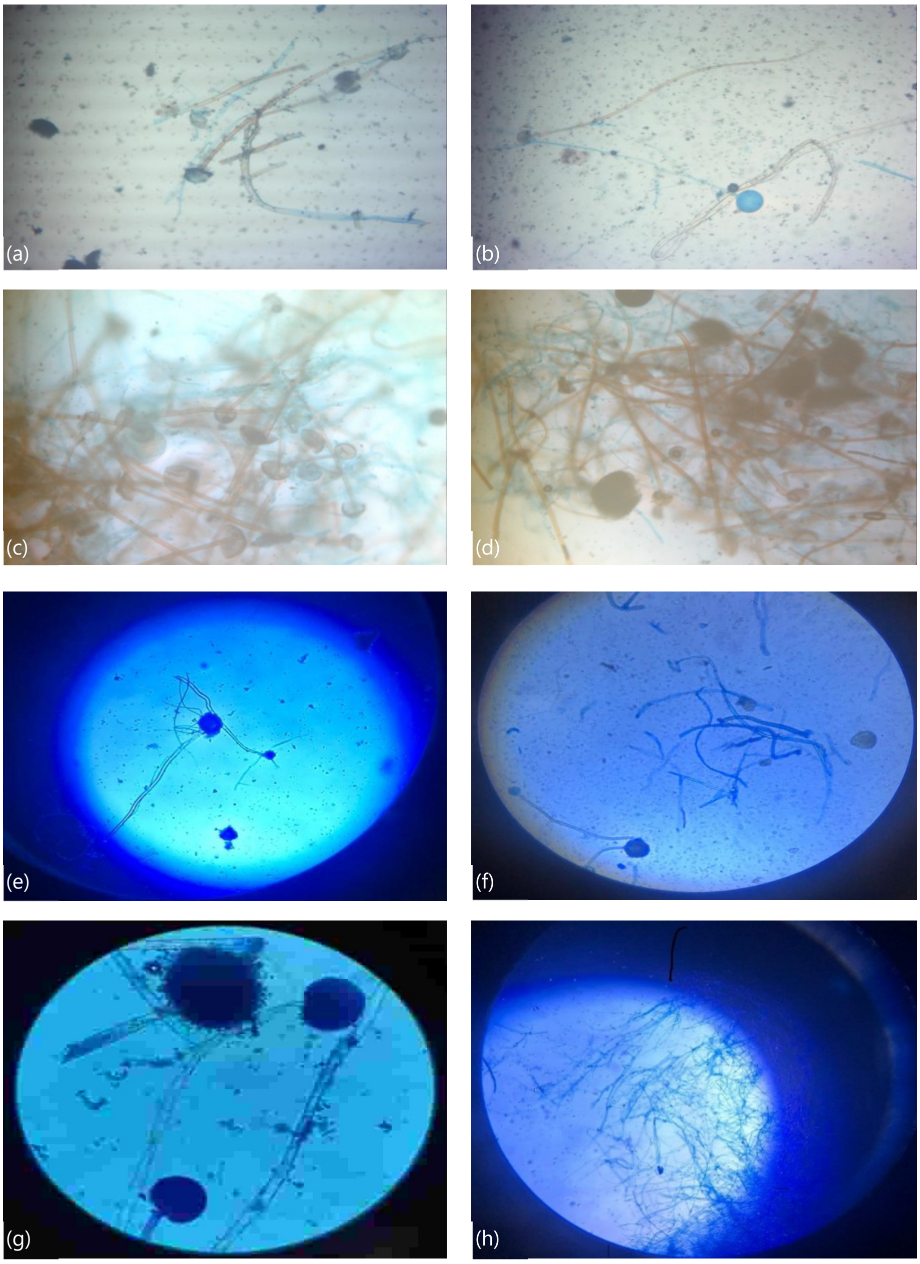

Fig. 1(a-h): Microscopic features of the isolated fungal pathogens, (a) *Candida albicans*, (b) *Rhizopus stolonifer*, (c) *Mucor hiemalis*, (d) *Rhizopus oryzae*, (e) *Aspergillus flavus*, (f) *Aspergillus fumigatus*, (g) *Aspergillus niger* and (h) *Cephalosporium acremonium*

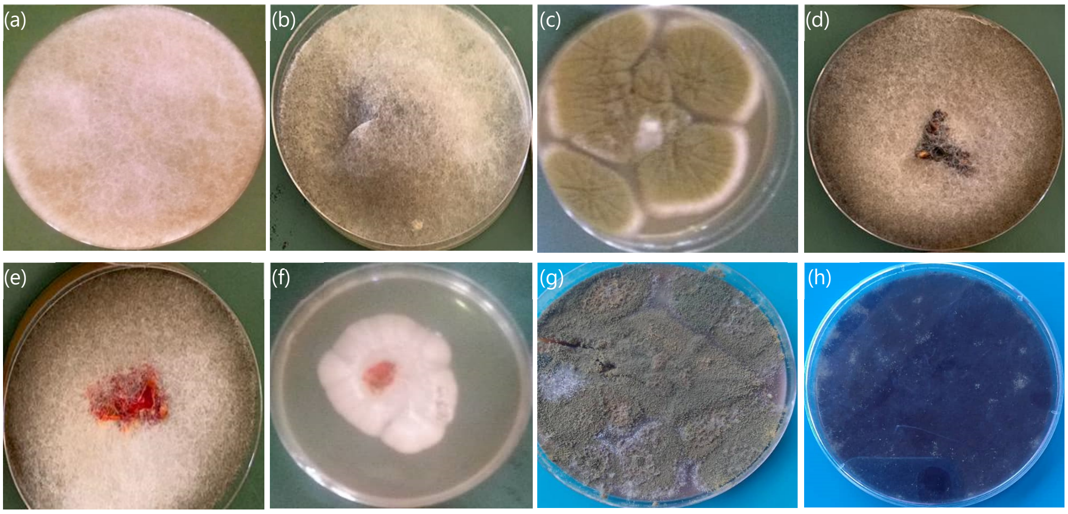

Fig. 2(a-h): Colonial features of the isolated fungal pathogens, (a) *Cephalosporium acremonium*, (b) *Rhizopus stolonifer*, (c) *Aspergillus flavus*, (d) *Rhizopus oryzae*, (e) *Mucor hiemalis*, (f) *Candida albicans*, (g) *Aspergillus fumigatus* and (h) *Aspergillus niger*

crops like peanuts and corn; *Aspergillus fumigatus* is an opportunistic pathogen that poses a risk to immunocompromised individuals; and *Aspergillus niger*, found on decaying organic materials, is industrially significant for enzyme production. Lastly, *Cephalosporium acremonium* is noted for its production of cephalosporins, antibiotics crucial in treating bacterial infections (Fig. 1a-h).

The fungal colonies exhibited distinct morphological characteristics. *Cephalosporium acreminium* displayed cottony to fluffy colonies, transitioning from white to pale yellow and eventually to grayish or brown with age, with a smooth or velvety surface and a pale yellow or cream reverse. *Rhizopus stolonifer* grew rapidly, forming initially white colonies that darkened to grayish-black or brown as sporangia developed, with a fluffy, cotton-like texture and a dark brown to black reverse. Similarly, *Rhizopus oryzae* produced colonies with a cotton-like texture, initially white and later turning grayish-black due to dark sporangia, with a fluffy and granular surface and a dark brown or black reverse. *Aspergillus flavus* formed flat, velvety colonies that appeared yellow-green to olive-green, becoming powdery or granular as they matured, with a pale yellow or cream reverse. *Aspergillus fumigatus* showed greenish-gray to bluish-green colonies with a velvety texture, turning powdery with maturity and a pale or cream reverse. *Mucor hiemalis* exhibited fluffy, cotton-like colonies that transitioned from white to grayish or brown with age, with a smooth surface and a pale or cream reverse. *Candida albicans* formed creamy white to off-white colonies with a smooth or slightly wrinkled surface, occasionally appearing moist or paste-like and a cream-colored or white reverse. These observations highlight the diversity in colony morphology among fungal species (Fig. 2a-h).

DISCUSSION

The findings of this study of eight fungal pathogens, which include *Cephalosporium acremonium*, *Mucor hiemalis*, *Rhizopus oryzae*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifer* and *Candida albicans* isolated from sweet pepper in Sokoto State during the dry season, are consistent with the findings of Shehu *et al.*[14], who isolated *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium oxysporum*, *Fusarium equiseti* and *Fusarium culmorum* during the rainy season in 2019 in their study of isolation, phenotypic characterization and molecular identification of fungal pathogens of sweet pepper fruit in Sokoto State, Nigeria. The findings of this research revealed that *Aspergillus niger* and *Aspergillus fumigatus* were both incriminated as pathogenic fungi of sweet pepper in Sokoto State in both the rainy and dry seasons.

Cephalosporium acremonium in this study displayed white to tan to rose-colored colonies with aerial hyphae. This is in line with the descriptions of Glenn *et al.*[15], who examine the taxonomy of *Acremonium* species and their phylogenetic relationships. Their study aligns with the observation that *Acremonium* species, can form colonies that range from white to tan to rose-colored. This morphological feature is commonly noted in *Acremonium* and is reflected in the research, which provides a basis for the variation in colony color due to genetic and environmental factors.

Glenn *et al.*[15] discuss the typical growth patterns of *Acremonium* species, which include the formation of aerial hyphae as the colonies mature. This is in direct alignment with your observation that *Cephalosporium acremonium* eventually develops white aerial hyphae, a key feature in the colony's progression as it matures.

The paper by Glenn *et al.*[15] also describes the conidia (spores) of *Acremonium* species, noting that they are typically single-celled, clear and elliptical. The description of the spores being held together in a ball until they are broken loose is consistent with the typical spore formation observed in *Cephalosporium acremonium*.

The findings of this research regarding a cottony, rapid-growing colony that fills a Petri dish in 3 to 5 days in *Mucor hiemalis* align with the findings of Thakur *et al.*[16], who mentioned that *Mucor hiemalis* is a fast-growing fungus, that fills a Petri dish in 3 to 5 days. This characteristic is common in many species of *Mucor*, which are known for their vigorous growth under appropriate conditions.

The study focuses on the production of L-asparaginase, an enzyme produced by the fungus, which is often linked with robust fungal growth, as the fungus needs to develop large mycelial structures to secrete enzymes efficiently.

Lebreton *et al.*[17] described *Mucor* species as fast-growing with fluffy, white to grayish colonies that spread quickly. The microscopic characteristics of clear, non-septate hyphae with large spherical sporangia are consistent with descriptions by Abdelhalim *et al.*[18], who reported that *Mucor* species produce non-septate, hyaline hyphae with large, spherical sporangia containing sporangiospores, but without rhizoids.

Rhizopus oryzae (also known as *Rhizopus arrhizus*) forms gray to brown to black colonies that fill a Petri dish in 2 to 3 days, which is corroborated by Abdelhalim *et al.*[18], who described the species as producing rapidly growing colonies that are initially white, turning grayish to blackish with age and characterized by the presence of rhizoids. The microscopic characteristics in this study, which show clear spores in sporangium with coenocytic hyphae, are consistent with the description by Cotado-Sampayo *et al.*[19], who noted that *Rhizopus arrhizus* produces large, multi-spored sporangia with non-septate hyphae and rhizoids.

The *Aspergillus flavus* colonial characteristics observed as bright yellow-green colonies are in agreement with the findings of Klich[20], who reported that *A. flavus* typically forms colonies with a distinct yellow-green appearance, which is an important diagnostic feature. Klich[20] noted that *A. flavus* produces biseriate conidial heads with roughened conidia as microscopic features.

Aspergillus niger colonial characteristics, which include flat, compact colonies that turn black, align with the observations by Visagie *et al.*[21] and are further supported by Klich[20], who described *Aspergillus niger* as having colonies that rapidly become black due to conidia production. The microscopic characteristics description of one-celled spores radiating from a swollen base is consistent with findings by Klich[20], who noted that *A. niger* produces conidial heads with radiating phialides and roughened dark conidia.

Aspergillus fumigatus, which forms green to dark green colonies, is supported by Latge[22], who reported that *Aspergillus fumigatus* forms velvety, dark green colonies that are characteristic of this species. The microscopic characteristics observed of long, globose-to-prolate conidia heads with hyaline conidiophores in this study are consistent with the findings of Lew[23], who noted that *A. fumigatus* has short conidiophores with a smooth surface and produces chains of conidia.

Rhizopus stolonifer colonial characteristics, with colonies turning gray to yellowish-brown over time, are corroborated by the descriptions from Thakur[16], who reported that *Rhizopus stolonifer* forms colonies that are initially white and cottony, turning grayish to brownish with age. The microscopic characteristics description of mycelium with black sporangia containing sporangiospores in *Rhizopus stolonifer* matches the observations of Abdelhalim *et al.*[18], who noted that *R. stolonifer* produces large sporangia on stolons, with characteristic black sporangiospores.

In *Candida albicans*, the colonial characteristics of white, creamy, smooth colonies are supported by Calderone and Fonzi[24], who described *Candida albicans* as forming smooth, creamy white colonies that are easily identifiable on Sabouraud agar. The oval to elongated yeast cells with budding formation observed in this study is consistent with the findings of Crampin *et al.*[25], who reported that *C. albicans* typically exhibit yeast cells with characteristic budding and pseudohyphae formation.

CONCLUSION

The study identified a diverse range of fungal species, including *Acremonium*, *Mucor hiemalis*, *Rhizopus oryzae*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifer* and *Candida albicans*, with varying growth patterns and morphological characteristics essential for accurate identification. Rapid-growing fungi like *Rhizopus* and *Mucor* highlight the need for regular environmental monitoring in agriculture and healthcare settings to prevent outbreaks. Future studies should focus on molecular techniques like PCR and sequencing for precise identification, as well as the pathogenic potential, antimicrobial resistance and ecological interactions of these fungi to better understand their roles and control mechanisms.

SIGNIFICANCE STATEMENT

Sweet pepper (*Capsicum annuum* L.) is a vital horticultural crop in Sokoto State, Nigeria, serving as a key source of income and nutrition for local farmers. This study investigates how seasonal changes influence the prevalence and diversity of fungal pathogens affecting sweet peppers, which has a direct bearing on crop yield and quality. Understanding these variations provides critical knowledge for developing season-specific management strategies, ultimately enhancing productivity and minimizing crop losses.

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