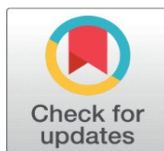
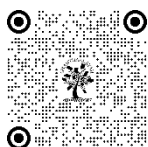


IMPACT OF MYCOFLORA ON RICE QUALITY AND STORAGE IN MADHUBANI DISTRICT

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ABSTRACT

Bioassays provide a reliable method for evaluating air quality through the examination of different bioparticles, including their occurrence, absence, quantity, distribution, shape, and biochemical properties. Bioparticles, including pollen, spores, bacteria, and other microbes, are crucial indicators of environmental conditions. These particles elicit responses from sensitive species of plants and animals, as well as humans, which offer valuable insights about air quality. Researchers can assess the environmental health of specific places by accurately measuring and establishing the habitat reactions and sensitivity of biological species. Within this context, there is a special emphasis on fungal spores due to their crucial role as environmental bioparticles that have well-documented effects on health. These spores are widely recognized for their capacity to induce dermatological, pulmonary, and cardiac ailments, as well as allergy symptoms in humans. Fungal spores have negative impacts not just on human health but also on other species and ecosystems. In order to investigate these concerns, an initial investigation was carried out on the presence of airborne fungi in rice mills situated in Madhubani. Rice mills provide an environment conducive to the growth and spread of fungi due to the processing of vast amounts of organic waste. The objective of this study was to categorize the many types of fungal spores found, measure their abundance in the atmosphere, and analyze their possible effects on both the mill workers and the neighboring population. The findings offer useful insights into the management of air quality and contribute to the development of methods to reduce the health concerns linked to exposure to airborne fungus spores.

Keywords: Fungal Spores, Rice Mill, Allergens



1. INTRODUCTION

Studies on fungal contamination in agricultural processing and storage environments repeatedly identify *Aspergillus* species as predominant, particularly in granaries, warehouses, and especially rice mills [1], [2]. Among these, *Aspergillus flavus* often shows two distinct seasonal peaks: the first from September to November (post-monsoon) and the second from May to June (pre-monsoon). This seasonal variation closely correlates with climatic parameters like humidity and temperature that favor fungal growth and reproduction [2], [3]. Conversely, *Cladosporium* species are predominantly observed in winter months, suggesting their adaptability to cooler and less humid environments [4].

Besides these dominant genera, several other fungi contribute significantly to the fungal diversity of these environments, such as *Rhizopus*, known for rapidly spoiling stored grains; *Curvularia*, which frequently occurs on plant debris and triggers allergies; *Aspergillus versicolor* and *Aspergillus fumigatus*, known producers of hazardous

mycotoxins; *Epicoccum nigrum*, common in soil and decaying organic material; and *Alternaria*, a known plant pathogen and allergen [5].

Previous research conducted by Pugalmaran and Vittal highlighted the complex and varied fungal communities found in grain storage facilities, influenced by storage conditions, geographic location, and grain types [6]. This diversity emphasizes the importance of understanding fungal species distribution within agricultural processing environments.

The current research aimed to assess fungal diversity in rice mills of Madhubani, an area characterized by unique climatic conditions conducive to fungal proliferation. Specifically, the study sought to identify the fungal species present, evaluate their seasonal variation, and examine their implications for stored grain quality and the health of mill personnel [7], [8]. Findings from this investigation enhance our understanding of fungal ecology within rice mills and provide critical insights for developing strategies to mitigate fungal contamination and associated health risks in grain storage and processing industries [9].

2. MATERIALS AND METHODS

The sampling process for the study was undertaken with great attention to detail, both inside and around the atmospheric conditions of rice mills in the Madhubani district, specifically targeting industrial locations. In order to obtain a thorough and detailed understanding of airborne fungal spores along with other microorganisms, air samples were methodically gathered at frequent and consistent time intervals. The collecting strategy utilized the settling plate culture method, a widely recognized technique for detecting airborne microorganisms [9], [10].

In order to minimize bacterial contamination and maintain the accuracy and purity of the samples, acetic acid was used as a preventive step. To capture airborne particles, Petri dishes with a 9 cm diameter were placed strategically at a height of 1 meter above the ground. Each dish contained 20 ml of the chosen growth material, Potato Dextrose Agar (PDA). In each instance, the exposure occurred for a consistent duration of 15 minutes. Following exposure, the Petri dishes were meticulously packed and transferred to the laboratory in a controlled environment [9].

Upon arrival at the laboratory, the specimens were placed in a controlled environment at a consistent temperature of 30°C for a duration of 5 to 7 days. This incubation was done to facilitate the ideal development and recognition of fungal colonies [7], [9].

In order to improve the dependability and thoroughness of the investigation, samples were gathered from multiple regions inside the industrial zones, including numerous sites within the rice mills around the Madhubani district. Every sample was carefully dried and stored in suitable circumstances prior to undergoing microscopic analysis for the purpose of identifying and characterizing the fungus species that were present. To cultivate and isolate fungi, three sets of media plates comprising Potato Dextrose Agar (PDA) were created as duplicates. The media plates were fortified with streptomycin and penicillin, each at a dose of 50 mg, to hinder the growth of bacteria and guarantee the exclusive growth of fungal species [9], [11]. Sterilized containers were used to transport the prepared media plates to the sample sites in order to preserve their integrity and prevent any possible contamination during the sampling process.

This meticulous sampling and analytical technique was developed to gain a comprehensive understanding of the fungal variety found in the rice mills of Madhubani, particularly in industrial areas, and to evaluate the potential health hazards linked to airborne fungal spores in these settings. The results of this study will enhance our understanding of environmental mycology and aid in the creation of methods to reduce the effects of airborne fungus on public health and industrial activities [7], [9], [11].

3. RESULTS AND DISCUSSION

As part of this thorough investigation, a wide-ranging survey was carried out to evaluate the variety of fungi present in and around the rice mill environment. The investigation produced a noteworthy result, as 75 different fungus species were identified, totaling 300 fungal colonies. The researchers carefully categorized these fungal species into 38 distinct fungal genera, each playing a role in the varied fungal ecosystem identified in the study. This work offers significant insights into the fungal ecology seen in industrial environments, with implications for their own safety, health, and the control of fungal contamination [7], [9].

1) Zygomycotina Division

The Zygomycotina division, also referred to as Zygomycetes, is distinguished by its hyphae that lack septa and its tendency to proliferate rapidly. A total of 23 fungal colonies, representing four separate genera, were isolated from this division, comprising four fungal species. Zygomycotina species are mostly recognized for their function in the breakdown of organic matter, which aligns with their occurrence in a rice mill setting, where organic residues are abundant [7], [11]. The identified species were:

- **Choanephora cucurbitarum:** This species is frequently found in close proximity to decomposing plant matter, especially in agricultural environments. The species' modest contribution of 0.23% implies that it does not have a dominant position in this habitat. However, its presence is noteworthy as it signifies the ongoing decomposition process in the mill [7].
- **Mucor hemalis:** Mucor hemalis, a species commonly found in soil and decomposing organic materials, exhibited a substantially larger contribution of 1.49%. This indicates that this particular species is more commonly found in the rice mill setting, likely because there is a high amount of organic material available for it to grow on [3], [7].
- **Rhizopus oryzae:** Rhizopus oryzae, which is well-known for causing food spoilage, especially in starchy substances, was found to be moderately present, contributing 0.46%. The presence of this substance is especially remarkable in a rice mill environment, where there is a high occurrence of residues that are rich in starch [7].
- **Syncephalastrum racemosum:** This species had the highest proportion of representation within the Zygomycotina division, accounting for 33.33%. Syncephalastrum racemosum is notable for its enzymatic production ability, which could potentially contribute to the degradation of organic matter in the mill [7], [11].

2) Ascomycotina Division

The Ascomycotina division, also known as Ascomycetes, is characterized by the presence of asci, which are sac-like structures that house spores. The study involved the isolation of six fungal species belonging to the Ascomycotina division. A total of 20 fungal colonies were obtained, which represented five different genera. Ascomycetes exhibit a wide range of ecological functions, such as being plant pathogens, saprophytes, and symbionts [5], [7]. The species discovered in this investigation are as follows:

- **Chaetomium globosum:** This species exhibits cellulolytic properties, enabling it to enzymatically degrade cellulose, a prevalent constituent of plant matter. The existence of this substance, which accounts for 0.46% of the total, suggests its involvement in the breakdown process that occurs in the mill [7].
- **Emericella nidulans:** Emericella nidulans accounted for the largest proportion, 0.57%, within the Ascomycotina division. This species is highly esteemed for its capacity to generate secondary metabolites, a number of which possess economic uses. The existence of it indicates the possibility of biochemical interactions occurring within the mill environment [5].
- **Eupenicillium javanicum and Eupenicillium purpurogenum:** Both species are classified under the genus Eupenicillium, closely related to the widely recognized Penicillium. Their relative contributions of 0.46% and 0.34% emphasize their importance in the natural process of disintegration. These species are renowned for their ability to produce antibiotics. However, in this particular context, their primary function is associated with causing spoilage and breaking down organic waste [7].
- **Pleospora herbarum:** Pleospora herbarum, commonly found on decomposing plant matter, exhibited a negligible contribution of 0.23%. This species is commonly linked to plant infections; however, in this particular setting, it probably aids in the decomposition of plant leftovers [7].
- **Thielavia terricola:** This species, present in the soil, also accounted for 0.23% of the total fungal population. The presence of soil-derived fungi in the rice processing plant environment highlights their significance, since they are likely to interact with the organic components found there [7].

3) Anamorphic Fungi

The Anamorphic Fungi, sometimes referred to as Deuteromycota or defective fungi, exhibited the highest level of diversity among the groups examined in this study. There were a total of 61 species, belonging to 27 genera, that were identified, resulting in a total of 300 fungal colonies. Anamorphic fungi are distinguished by their absence of a recognized

sexual reproductive stage and frequently play diverse ecological roles, such as saprophytism and pathogenicity [2], [4], [7]. The species that have been isolated are:

- **Cladosporium cladosporioides and Cladosporium sphaerospermum:** These two species had the highest occurrence, accounting for 16.22% and 15.42% of the total, respectively. Cladosporium species are frequently present in both indoor and outdoor settings and are recognized for their allergenic properties. Their significant presence signifies their capacity to withstand and adjust to the conditions of the rice mill surroundings, where they presumably flourish by consuming organic dust and remnants [1], [2].
- **Aspergillus species:** Multiple species of Aspergillus were detected, namely Aspergillus flavus (2.76%), Aspergillus niger (5.98%), Aspergillus fumigatus (3.79%), and Aspergillus versicolor (1.84%). The Aspergillus genus is renowned for its capacity to generate mycotoxins, which can provide substantial health hazards to both workers and consumers. The occurrence of such organisms in the rice mill is worrisome, especially in relation to food safety and worker health [2], [3], [7].
- **Curvularia species:** Curvularia oryzae and Curvularia lunata var. aerea were two of the species that were identified, with respective contributions of 1.72% and 6.21%. These species are recognized for their connection with plant diseases, especially in cereals, which makes their existence in a rice mill significant. They can potentially contribute to the deterioration of stored grains, impacting both their quality and safety [7].
- **Penicillium species:** Additionally, many species of Penicillium were identified, such as Penicillium chrysogenum, Penicillium notatum, and Penicillium lilacinum. These species are recognized for their involvement in the deterioration of food goods, as well as their ability to produce antibiotics. Their limited representation in the present investigation, with contributions that vary from 0.11% to 0.34%, indicates that they are not the prevailing species in this habitat, but they may still have an impact on the degradation processes [1], [7].
- **Nigrospora oryzae and Alternaria alternata:** These species, which account for 2.76% and 2.30% of the total, are frequently linked to plant pathology. Their existence in the rice mill setting suggests the possibility of plant material pollution and decay, which could affect the quality of grain that was stored [5], [7].

4) Mycelia Sterilia

The Mycelia Sterilia group, comprising fungi that lack the ability to form identifiable spores, posed a distinctive obstacle in our investigation [7]. Three species were isolated, totaling 19 fungal colonies, and were categorized according to the hue of their mycelia:

- **Mycelia sterilia (White):** This particular variant had the greatest prevalence among the Mycelia sterilia group, accounting for 1.15% of the overall fungal population. The presence of the white mycelium suggests that it may have a saprophytic function in decomposing organic matter within the mill.
- **Mycelia sterilia (black) and Mycelia sterilia (ash):** The variations exhibited contributions of 0.69% & 0.34%, which were classified as moderate and minimal, respectively. Black mycelium is frequently linked to the synthesis of melanin, which can offer defense against environmental pressures, whereas the mycelium that appears ash-colored may indicate a distinct ecological adaptation.

5) Unknown Fungal Species:

During this study, a fungal species that could not be recognized was found. It was portrayed by a single colony and accounted for 0.11% of the total fungal diversity. The existence of an unidentified species underscores the possibility of uncovering novel or uncommon fungi in industrial settings like rice mills. Additional investigation, utilizing DNA sequencing techniques, may be necessary to fully categorize this species and comprehend its ecological function [7], [9].

6) Percentage Contribution and Implications:

The study's findings about the proportionate contributions of individual fungal species throughout the year offer useful insights into the changing patterns of fungal communities in the mill's environment. The prevalence of particular species, such as Cladosporium cladosporioides and Syncephalastrum racemosum, suggests their capacity to flourish in the specific environmental conditions present in the mill. The existence of mycotoxin-producing species, specifically belonging to the Aspergillus and Penicillium genera, highlights the significance of supervising and managing fungal contamination in order to safeguard the well-being of workers and the integrity of stored products[1], [2].

The extensive record of fungal species found in the rice mills in the Madhubani area enhances our knowledge of fungal ecology in industrial environments. The study's results have important consequences for public health, namely in relation to the control of airborne fungal spores. These spores can be a threat to both human health and food safety. The detection of various fungal species, including potentially detrimental ones, highlights the necessity for continuous surveillance and the adoption of efficient fungal control measures to guarantee the safety and excellence of stored grains along with other agricultural commodities [3], [7], [9].

4. CONCLUSION

The results of this study largely correspond with earlier research conducted in comparable settings, strengthening the reported patterns of fungal diversity in rice mills. Desai et al. (2003) discovered the existence of *Cladosporium* species in rice mill's environments in India. This discovery aligns with the current study, which also found *Cladosporium cladosporioides* and *Cladosporium sphaerospermum* to be among the most commonly found species. In a similar manner, Lugauskas et al. (2004) observed the existence of *Mucor* species in the air of grain mills, accounting for 1.3% of all isolates. This aligns with our own findings of *Mucor hemalis* making a significant contribution.

In addition, the study conducted by Cetinkaya et al. (2005) on rice mills in Turkey found that *Cladosporium* species accounted for the highest percentage contribution (31.9%), followed by *Aspergillus* species (18.6%). Our investigation supports this conclusion, as we observed that *Cladosporium* and *Aspergillus* species were the most prevalent. Specifically, *Cladosporium cladosporioides* accounted for 16.22% and *Aspergillus niger* accounted for 5.98% of the overall fungal population. In our investigation, we found that *Penicillium* species were present, although in slightly lower amounts compared to the work by Cetinkaya et al. This suggests that *Penicillium* species are important in these habitats, contributing approximately 15.5%.

The consistent presence of these fungal taxa in rice mill environments, as shown in various geographical areas and research, highlights their widespread nature. The significant occurrence of *Cladosporium*, *Aspergillus*, and *Penicillium* species emphasizes the possible health hazards and spoilage issues linked to these habitats, primarily because certain species within these genera have the ability to produce mycotoxins.

Ultimately, the findings of this study not only correspond with prior research but also add to the expanding collection of knowledge regarding the fungal diversity in rice mills. The persistent occurrence of particular fungal genera in various studies highlights the necessity for ongoing surveillance and the enforcement of rigorous fungus management protocols in rice mills to reduce potential health hazards and guarantee the safety of stored products. Future studies should prioritize the identification of unfamiliar fungal species and the investigation of their functions within these habitats, together with the creation of specific approaches to control fungal contamination in rice mills.

CONFLICT OF INTERESTS

None.

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