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EXPLORING FUNGAL CONTAMINATION IN MARKETED MILLETS: A DIVERSITY STUDY

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Abstract:

Millets are a group of small-grained Cereals. They have been a part of the Indian food basket for hundreds of years. They are deeply ingrained in our food systems culture and traditions. Millets are recognized as climate-resilient and nutritionally rich cereals. However Post harvest handling, storage practices and open market conditions affects these grains to fungal contamination. The present study aims to explore the diversity and prevalence of fungal contamination in selected millets sold in open retail Outlets. Three millets viz; Bajra, kodo and ragi sold in the market of Bilaspur were studied for the presence of fungal flora within them. Collected samples were grown on the Potato dextrose agar medium and the fungal isolates grown over culture plates were purified by the method of single colony isolation. The fungi growing on the three millets were identified on the basis of their colony characteristics, morphological features and asexual reproductive characteristics. The result were identified as diversity of fungal species, predominantly belonging to the genera Aspergillus, Penicillium, Fusarium, Alternaria, Mucor, Rhizopus, Phoma and Trichoderma. The study highlights diversity of fungal flora in open market of Bilaspur, Chhattisgarh. It provides the need of regular monitoring, improve Post-harvest handling and Storage practices to control the Safety and quality of millet grains in markets. Besides the outcomes point out the essential role of mycological assessment with food safety regulations to reduce mycotoxin Contamination and protect public health while advancing millets as Sustainable and nutrient-rich crops.

Keywords: Millets, Fungal contamination, Fungal flora, Post harvest handling, Mycological assessment, Public health.

Introduction:

Millets are a distinct group of small-seeded cereals from the poaceae family, are an important food source for millions of people in Asia and Africa providing nutritional security and income opportunities for Small holder farmers (Gupta *et al*.,2017). These crops are widely grown in arid and semi-arid regions due to their resilience to drought, low input requirements and adaptability to marginal soils (FAO, 2012). Recently, millets have gained global interest because of their superior nutrition composition, including high levels of fibre Important amino acids, minerals (iron, Calcium, Zinc) and bioactive compounds with antioxidant properties (Saleh *et al*., 2013, Chandra *et al.,* 2016). Policy and research frameworks have been increasingly emphasized millets potential contribution to food and nutritional security particularly under Climate-resilient agriculture initiative promoted by the food and Agriculture organization (FAO, 2021).

Despite their agronomic and nutritional advantages millets are highly Susceptible to fungal Contamination during pre harvest, harvesting, transportation and Storage conditions. The favourable conditions of fungal growth are create by environmental factors such as high humidity, temperature fluctuations, insect Infestation and lack of Post harvest handling. (magan and Aldred, 2007). In the stored cereals and millets, species belonging to the genera Aspergillus, Fusarium and Penicilium are frequently isolated. (Pitt and Hocking, 2009; Kaur *et al.,* 2019). These Fungi are not only responsible for visible spoilage but also produce toxic Secondary metabolites known as mycotoxins. (Bennett & Klich, 2003).

Mycotoxin, including aflatoxins, fumonisins and ochratoxins are of major Public health concern due to their carcinogenic, hepatotoxic, nephrotoxic, immunosuppressive and teratogenic effect. (Bennelt and klitch, 2003; Kumar *et al.,* 2017). Aflatoxin B_1, Produced by Aspergillus flavus and A. parasiticus is classified as a group 1 Carcinogen by the International Agency for Research on Cancer (IARC, 2012). Chronic dietary exposure to mycotoxins through contaminated cereals poses significant risk especially in developing countries where regular enforcement and routine Surveillance may be limited (Williams *et al.,* 2004; Hell *et al.,* 2010).

Several Studies have documented fungal diversity and mycotoxin contamination in cereal grains; however Comprehensive assessments focusing spatially on marketed millets particularly those sold in open market system remain limited (Kumar *et al*., 2008). Variation in environmental conditions, cultivation Practices, and market infrastructure and supply Chain management influence the composition of fungal flora in millets (Kumar *et al*., 2008; Perrone and Susca, 2017). Therefore systematic investigation of mycoflora diversity in marketed millets is necessary for risk assessment, quality assurance and Formulation of targeted risk reduction strategies.

In this context, the present study focuses on the fungal association of marketed millets with an emphasis on their diversity. This study attempts to generate baseline data on the occurrence and distribution patterns of fungi by identifying and characterizing the associated fungal flora. Such knowledge is essential for designing sustainable interventions to improve shelf life, reduce economic losses and ensure the availability of safe and high quality of millets to consumers as climate-resilient and nutrient-rich crops.

Materials and Methods:

Culture Media: Potato Dextrose Agar (PDA) medium with the specified composition was used for the isolation of fungal flora present in the millets. The composition, pH, and supplements of the medium were maintained as required. All chemicals used were of analytical grade and obtained from various commercial sources.

Collection of Millet samples: Samples of the three millets were collected from Shanichari market of the Bilaspur city. These were kept in Polythene bags carried to the laboratory and kept inside deep freezers to minimize further Contamination.

Culture of Fungi: Before culturing, Samples were water soaked, grind and made in the form of paste by using little amount of Sterilized water. This was filtered by double layered muslin cloth. The filtrate was used as the source of fungi. The filtrate was diluted thrice by the method of serial dilution using 0.9% normal saline (NaCl). 0.2 ml of third dilution was Plated on the petriplates containing Potato dextrose agar Culture media, plates were incubated at 30·C continuously for five days, The Colonies were Counted.

Identification of fungal isolates: The Petri plates obtained after incubation revealed mixed colonies of various fungal species growing on the Potato Dextrose Agar medium. These colonies differed in their colour, texture, margin, elevation, and growth pattern, indicating the presence of diverse fungal flora associated with the millet samples. To obtain pure cultures, individual colonies were carefully selected and purified using the simple colony isolation technique, followed by repeated sub-culturing. This procedure ensured the removal of contaminating organisms and facilitated the establishment of axenic fungal cultures. The purified isolates were maintained on PDA slants for further examination. Identification of the fungal species was performed using standard mycological methods based on macroscopic and microscopic characteristics. Macroscopic features such as colony colour, surface appearance, reverse pigmentation, and growth rate were recorded. Microscopic examination was carried out by preparing lacto phenol cotton blue mounts to observe mycelial structure, septation, and hyphal arrangement. Special emphasis was given to the morphology of conidiophores, including their branching pattern and size. The arrangement, shape, and surface characteristics of conidia were also carefully examined. The identification of fungal isolates was confirmed by comparing the observed characteristics with standard mycological manuals and keys as described by Gilman (1966), Barnett and Hunter (1972), and Nagmani (2013).

Results and Discussions:

The findings of the present study revealed a rich and diverse assemblage of fungal species associated with millets collected from open market conditions. Examination of both infected and apparently healthy samples showed that fungal colonization occurred at varying frequencies among different millet types. These variations may be attributed to factors such as storage duration, grain structure, moisture content, and handling practices adopted in open markets. (Magan and Aldred, 2007; Pitt and Hocking, 2009). Isolation and purification on Potato Dextrose Agar (PDA) led to the recovery of multiple fungal genera, indicating extensive fungal contamination during post-harvest handling, storage, and marketing of millets

The fungal flora identified included *Aspergillus flavus, Aspergillus fumigatus, Aspergillus oryzae, Aspergillus tamarii, Aspergillus niger, Fusarium solani, Penicillium chrysogenum, Penicillium citrinum, Alternaria alternata, Mucor* species, *Rhizopus* species, *Cladosporium* species, *Phoma* species, and *Trichoderma* species. (Gilman, 1966; Barnett and Hunter, 1972; Nagmani *et al*., 2013).

Colonies of various fungal species were also count for the purpose of analyzing the fungal load on the millets.

The data has been presented in the table 1

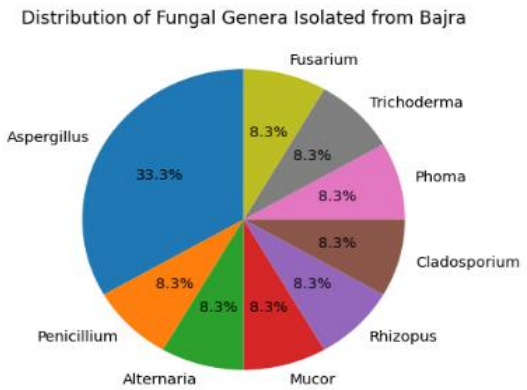
Sr. No.	Name of Fungal species	Number of Colonies		
		Bajra	Kodo	Ragi
1	*Aspergillus flavus*	14	12	11
2	*Aspergillus fumigatus*	12	10	09
3	*Aspergillus oryzae*	11	-	06
4	*Aspergillus tamarii*	-	14	08
5	*Aspergillus niger*	13	07	09
6	*Fusarium solani*	12	-	-
7	*Penicillium chrysogenum*	11	10	02
8	*Penicillium citrinum*	-	12	19
9	*Alternaria alternata*	10	-	-
10	*Mucor* species	08	12	-
11	*Rhizopus* species	09	-	10
12	*Cladosporium* species	11	14	-
13	*Phoma* species	08	-	06
14	*Trichoderma* species	06	06	05
Total		125	97	85

From the above data presented in table fungal load on the three millets were calculated to be 125 in bajra, 97 in kodo and 85 in ragi. Among the isolated fungi, species of the genus *Aspergillus* were predominant, suggesting their strong adaptability to dry food commodities such as millets. The frequent occurrence of *A. flavus* and *A. niger* is of particular concern, as these species are well known for their ability to survive under low moisture conditions and for their association with mycotoxin production. (Bennett & Klich, 2003; Kumar *et al*., 2017). The presence of *A. fumigatus* further indicates possible health risks, as it is considered an opportunistic pathogen. *A. oryzae* and *A. tamarii*, though commonly associated with fermentation processes, also reflect improper storage environments that favour fungal growth.

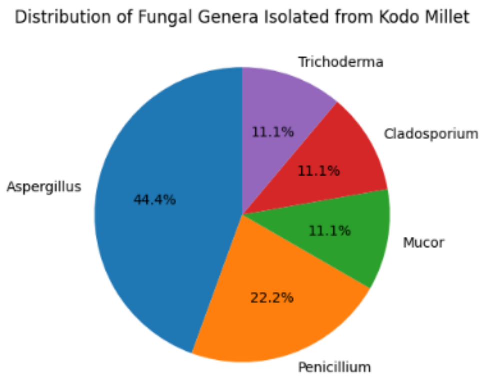
The detection of *Penicillium chrysogenum* and *Penicillium citrinum* highlights the role of storage conditions in fungal contamination, as these species are commonly associated with stored grains and cereals (Perrone and Susca, 2017). Similarly, the isolation of *Fusarium solani* is significant due to its association with grain spoilage and potential toxin production (Kaur *et al.,* 2019). The presence of *Alternaria alternata* suggests field-level contamination, as this fungus is often linked with pre-harvest infections and environmental exposure (Nagmani *et al.,* 2013).

Zygomycetous fungi such as *Mucor* and *Rhizopus* species were also identified, indicating high humidity and poor aeration during storage or transportation (Pitt and Hocking, 2009). These fast-growing fungi are commonly associated with spoilage and reduced shelf life of food grains. The occurrence of *Cladosporium* and *Phoma* species further supports the influence of environmental and handling factors, as these fungi are widely distributed in air, dust, and soil. The isolation of *Trichoderma* species, though generally considered non-pathogenic and sometimes beneficial, reflects the diverse microbial ecology present in marketed millets.

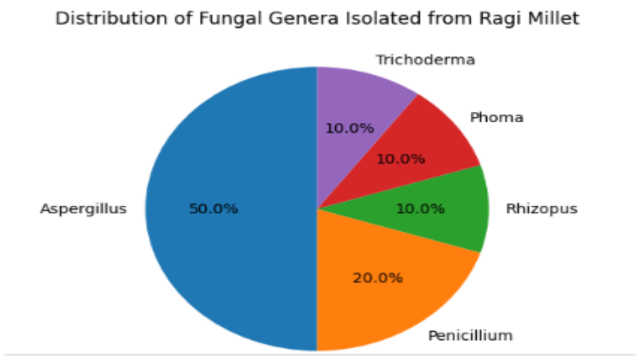
Overall, the results demonstrate that marketed millets contained a diverse fungal population, dominated by storage-associated fungi, particularly *Aspergillus* and *Penicillium* species. The findings emphasize the need for improved post-harvest management practices, proper storage conditions, and regular monitoring of fungal contamination to ensure the safety and quality of millets available to consumers. The presence of potentially toxigenic fungi highlights the importance of adopting strict food safety measures to minimize health risks associated with the consumption of contaminated millets.Similar observations have been reported by earlier workers, including Gilman (1966) and Barnett and Hunter (1972), who documented the frequent occurrence of storage-associated fungi such as ***Aspergillus***, ***Penicillium***, and ***Fusarium*** species in cereal grains. Further, Nagmani *et al.* (2013) reported the dominance of ***Aspergillus flavus***, ***Aspergillus niger***, ***Penicillium chrysogenum***, and ***Alternaria alternata*** in marketed food grains under improper storage conditions, which strongly supports the findings of the present investigation.



(a)



(b)



(c)

Figure- Pie diagram showing the prevalence of different fungal genera on the three millets

(a). Bajra (b). Kodo (c). Ragi

In bajra Aspergillus was the dominant genus, contributing 33.3% (4 species), all other genera Penicillium, Alternaria, Mucor, Rhizopus, Cladosporium, Phoma,Trichoderma, and Fusarium each contributed 8.3% (1 species each). This indicates a strong predominance of *Aspergillus* species in bajra samples compared to other fungal genera.

In kodo millet Aspergillus was the dominant genus, accounting for 44.4% (4 species out of 9). Penicillium contributed 22.2% (2 species). Mucor, Cladosporium, and Trichoderma each represented 11.1% (1 species each), Alternaria, Rhizopus, Phoma, and Fusarium were not detected in kodo millet samples.

In ragi millet Aspergillus was the most dominant genus, accounting for 50.0% (5 species). Penicillium contributed 20.0% (2 species), Rhizopus, Phoma, and Trichoderma each accounted for 10.0% (1 species each), Alternaria, Mucor, Cladosporium, and Fusarium were not detected in ragi millet samples.

In conclusion the present study demonstrates the substantial diversity and occurrence of fungal contamination in marketed millets collected from open retail outlets. A wide range of fungal genera, predominantly *Aspergillus*, followed by *Penicillium*, *Rhizopus*, *Mucor*, *Cladosporium*, *Phoma*, *Trichoderma*, *Fusarium*, and *Alternaria*, were isolated from bajra, kodo, and ragi samples, with notable variations in their distribution among different millet types. The dominance of *Aspergillus* species across all millets indicates their strong adaptability to storage and market conditions, while the presence of other storage- and field-associated fungi reflects the influence of post-harvest handling, moisture content, and storage practices (Magan and Aldred, 2007; Pitt and Hocking, 2009). The detection of potentially toxigenic and spoilage fungi highlight the risk to food quality and consumer health. Overall, the findings emphasize the need for improved post-harvest management, hygienic handling, and proper storage conditions to minimize fungal contamination and ensure the safety and quality of millets available in open markets (Bennett and Klich, 2003; Kumar *et al.*, 2017; Williams *et al.,* 2004). Detailed investigation with regard to the damage, biochemical deterioration, hazards on human health and possible safety measures are needed in future.

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