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ORIGINAL ARTICLE

# Fungal Deterioration of Maize Grains in Bilaspur (Chhattisgarh), District in India

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## ABSTRACT

Maize (*Zea mays* L.) is a critical cereal crop in Chhattisgarh, contributing significantly to the agricultural economy. However, the region's hot and humid climate, combined with inadequate storage facilities, often leads to the rapid fungal deterioration of maize grains in a very short time. Deterioration of maize is mainly affected by moisture content, temperature, humidity, storage conditions, fungal growth, and insect pests. Fungal growth, mostly *Aspergillus flavus*, *Aspergillus niger* and *Fusarium* sp. in maize is facilitated by hot and humid conditions, posing a risk through mycotoxins production. To maintain high-quality maize for short and long term-storage, maize must be protected from weather, and the growth of microorganisms and pests. So far,the present investigation of fungal deterioration has been done. Random maize seed samples were collected from various places in Bilaspur (District) *viz.* Masturi, Kota, Ratanpur, Sipat, Sakri, and Bilaspur. The collected samples were stored individually in pre-sterile polyethene bags at room temperature until use. Five random maize samples were collected from each tahsil. Moisture, pH, Biochemical and mycological analysis were done. Moisture and pH of each sample were studied. Mycological analysis through the Agar Plate and Blotter paper methods was performed, and fungal species were identified morphologically. In the Agar plate method (unsterilized and sterilized method) 15 species and 12 species were found respectively similarly in the Blotter paper method in both unsterilized, sterilized method 14 species and 10 species were found respectively. Based on per cent occurrence on Agar plate method following species were found to be dominant *A. flavus, A. niger, Fusarium oxysporum*, *A. terreus, A.ochraceus*. In contrast, Blotter paper method revealed that *A. flavus, A. niger, F. oxysporum,* were found to be dominant.Their cultural characteristics were studied and identified by morphological observations. Biochemical analysis in terms of Carbohydrate and Protein content were performed, pathogenicity test of dominant fungal species was also done which revealed that *A. flavus, A. niger, F. oxysporum* caused loss in weight and percent seed germination. These following species also reduced carbohydrate and protein in comparison to the control set after 15 days of storage.The research highlights the role of temperature and moisture in fostering fungal growth, particularly *Aspergillus* and *Fusarium* species, which cause aflatoxin contamination. However, synthetic chemical pesticides are used to manage insect infestation and control mould development in maize, but their residues have been detected on the stored maize before consumption. Additionally, continuous use of synthetic chemical pesticides has led to the development of pathogen/pest resistance reducing their effectiveness. Residuals from synthetic fumigants could cause health hazards, environmental contamination, and loss of seed viability. Hence this investigates the primary factors contributing to maize grain deterioration in Chhattisgarh, including environmental conditions, pest infestations, and improper post-harvest handling and interventions to mitigate these issues.

**KEY WORDS:** Maize, fungal growth,postharvest losses, storage fungi, mycotoxin, synthetic fumigants

## INTRODUCTION

In many regions of the world, maize is one of the main sources of energy and protein needed to prepare various kinds of foods for humans. After rice and wheat, maize (*Zea mays* L.) is the third most produced grain globally and one of the main cereals. In addition to being a staple food for humans and cattle, maize is a significant commodity in global trade (Basappa, 2009). More than 200 million people consume maize in various forms as a staple food nowadays (Du Plessis, 2003). Every year, this rate of

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consumption increases. According to FAO forecasts (FAOSTAT 2012), the demand for maize from human and animal consumption will rise by around 300 million tons by 2030. It doesn't include the need for industrial applications (O'Gara, 2007). Additionally, it's believed that the world uses roughly 65% of its total maize production (Abassian, 2006). Because it has the largest genetic production potential of all the cereals, maize is referred to as the "Queen of cereals" globally. About 150 million hectares spread across 160 nations, with a greater diversity of soil, temperature, biodiversity, and management techniques, account for 36% (782 million metric tons) of the world's grain production. With 35% of the world's total production, the United States of America (USA) is the world's greatest producer of maize, which powers the US economy. The productivity in the USA is the highest at 1.02 times that of the global average (4.92 t $ha^{-1}$). On the other hand, India's average production is 2.43 t $ha^{-1}$ (FAO,2013).

In India, maize is a promising crop that is used extensively for human consumption (25%) animal feed (12%), poultry feed (49%), industrial products (primarily starch) (12%), and beverage and seed (1% each). A substantial amount of maize has also been exported from India to other nations in recent years.Presently, maize production in India stands at 26 million tons and is expected to attain about 45 million tons by 2030 (FCCI, 2018; Ramamurthy *et al.*, 2020). It is cultivated annually in India, though more than 80% is grown in rainy or *kharif* season (July to October). *Kharif* maize represents nearly 83% of the maize area, while *rabi* maize corresponds to 17% area. Typical proximate compositions of the main parts of the maize kernel (yellow dent corn).

Agriculture is counted as the chief economic occupation of the Chhattisgarh state. Attributable toChhattisgarh's agro-climate, maize is the second most important crop after rice in the state. Production and productivity were rising steadily in the Chhattisgarh region. About 80 percent of the population in the state is engaged in agriculture and 43 percent of the entire arable land is under cultivation (Sinha *et al.,* 2019). The people in this state plant maize for a variety of functions. Some grow it for commercial interests, while others use it for human consumptionand animal feeding. In Chhattisgarh, maize is typically planted in baadies (the space behind homes). Although it is often grown in all seasons, in this state, kharif is the best time to cultivate it (Dhruv *et al*., 2022). Different districts of Chhattisgarh like Bastar, Bijapur, Dantewada, Sukma, Gariyabhand, Kondagaon, Kanker, Korea, Korba, Surajpur, Balrampur, and Sarguja districts are the principal places where it is grown most frequently. These areas are predominantly tribe's region. In the last few years, the yield of maize has greatly increased in the districts of Balrampur, Surajpur, Surguja, Kanker, Bastar, and Jashpur, Bilaspur (Sinha *et al*., 2019). It has been reported that maize grains undergo pronounced biochemical and nutritional changes during storage (Morrison & Nelson, 1991). Abiotic factors (temperature, moisture, humidity, rain) and biotic factors (insects, pests, rodents, fungi) are the two primary groups of factors that determine storage losses (Rahman *et al*., 2012). Temperature and moisture content are the two most important variables influencing storage life. Moisture contents in grains or humidity cause dramatic changes in the acidity, pH, protein, starch quality (Zhang *et al.,* 2010). Hot, humid weather promotes the growth of fungi in maize (Egal *et al.*, 2005). Numerous researchers have observed that fungal infestation in maize causes color changes, reductions in nutritional content, and a drop in the amount and general quality of the grain. The two main fungus linked to grain storage, which includes maize, are *Fusarium* sp. and *Aspergillus flavus*. A serious risk to both humans and animals is posed by fungal growth in maize, as it produces mycotoxins, particularly aflatoxins. Direct exposure to mycotoxins could result in hepatotoxicity, genotoxicity, teratogenicity, nephrotoxicity and immunosuppression. In addition to lowering yield and product quality, the spread of aflatoxigenic fungi allowed hazardous metabolites to infiltrate the food chain.This present investigationaimsto record and discuss about the primary causes of maize deterioration in Bilaspur district ofthe state of Chhattisgarh, and bid solutions for mitigating the problems that have been found.

## METHODS

### Study Area

The experimental work for the isolation of seed-borne fungal pathogens of maize grain samples (Fig. 1) obtained from different tahsils of Bilaspur District) *viz:* Masturi, Kota, Ratanpur, Sipat, Sakri, and Bilaspur were carried out at the Natural Product laboratory in the Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh, India.

### Grain Moisture Content and pH

To determine moisture content, 10g sample from each maize variety was dried in an oven (100°C) till their weights became constant and the differences in weight were calculated from the following formula (Mandeel, 2005). For pH, a 1:10 sample: distilled water (w/v) suspension of each maize variety was prepared and mixed through gentle shaking. The pH of the sample was measured using a pH meter.

$$Moisture\ content\ (\%) = \frac{Fresh\ weight\ of\ maize\ grains - Dry\ weight\ of\ maize\ gains}{Fresh\ weight\ of\ maize\ grains} \times 100$$

## Isolation Using Agar Plating Method

Infected maize grains were surface sterilized with sodium hypochlorite ($NaClO_2$) after that theseeds were rinsed using sterile distilled water and dried on a sterile blotter paper for two minutes. Potato Dextrose Agar (PDA) was used in the fungalisolation (Hussain *et al.*, 2011). A maximum numberoffive seeds were plated on the sterile PDA and poured into each petri dish. Five seeds were placed equidistantly in each dish, had three replicates, and were incubated at 25°C at room temperature. Subculturing was also done using PDA to obtain pure cultures. Procedures were done in sterile working environments.

The occurrence frequency (OF) of mycobiota in maize samples will be calculated by formula:

$$Occurrence\ Frequency(\%) = \frac{Number\ of\ fungal\ isolates\ in\ each\ maize\ sample}{Total\ number\ of\ seed\ incubated} \times 100$$

## Isolation Using Blotter Paper Method

Three pieces of blotting paper Was sterilized using the conventional blotter approach by immersing them in ethyl alcohol, letting them dry, and then putting them inside a petri dish that Was sterilized. 5 mL of sterile water Was used to wet the blotting papers (ISTA, 1977; Agrawal 1981; De Tempe, 1987; Jain *et al.*, 2020). A total of hundred seeds without surface sterilized were tested for each variety. Ten seeds Were placed on three layers of moist blotting paper in each Petri dish. The Petri dishes Were incubated at 25 ± 1°C under 12/12 hrs light and darkness cycle for 7 days. Each seed Was observed under a microscope to record the presence of fungal colony and temporary slides Were prepared from the fungal colony for observation under compound microscope.

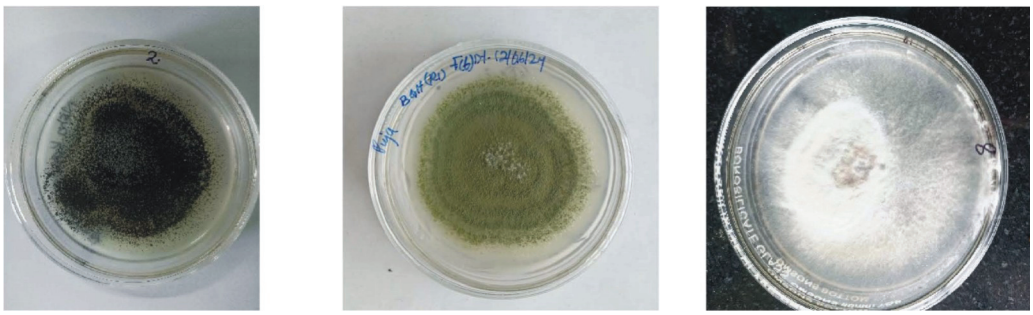
Each individual incubated seed was observed under microscope at 10x, 40x magnification to record the incidence of seed-borne fungi. Most of the associated pathogens were detected by observing their growth characters on the incubated seeds on blotter paper following the keys outlined by (Mathur & Kongsdal, 2003)and with the help of different books, manuals and publications. The results were presented as occurrence frequency for individual pathogen.

$$Occurrence\ Frequency(\%) = \frac{Number\ of\ fungal\ isolates\ in\ each\ maize\ sample}{Total\ number\ of\ seed\ incubated} \times 100$$

## Identification Using Microscopy

The process of identifying fungal seed-borne pathogensthat formed an overgrowth on maize grains was done using a compound microscope. Presence of characteristics of fruiting structures as done using spore color and colonization. The isolated fungi fruiting

Fig. 1. Deterioration of stored maize grains.



*A. niger* *A. flavus* *F. oxysporum*

Fig. 2. Dominant fungi isolated from Agar plate technique.

structures were examined after slide preparation. The seed-borne fungal pathogens were also identified using taxonomic features such as conidia and hyphae. Identification was done through manuals and slides preserved and kept in our Natural Product Laboratory.

## PathogenicityTest

The pathogenicity of identified fungi isolated from maize seeds was performed *in vitro* according to Koch's postulates by using 2-factorial experiment in Randomized Completely Block Design (RCBD) with 5-replicates. Seeds were surface sterilized with (Sodium Hypochlorite 13% v/v) followed by thorough rinsing with sterilized water. Five seeds were blotted on sterile filter paper and thereafter inoculated by soaking them in a homogenized mycelial suspension of the test fungus (*A. flavus*). Then, five seeds per replication were placed on 1% water agar (WA) plates and subsequently incubated at 28 ± 2°C for growth to check the fungal positivity (Hussain *et al.,* 2013).

A pathogenicity test on seedlings was conducted using 4 to 5 days old seedlings grown under aseptic conditions. Seedlings were aseptically transferred to slants of water agar (WA) in test tubes (1/tube). Five seedlings were inoculated per test tube and then incubated in the growth chamber at 25±2°C. The control samples were sprayed only with distilled water. The symptoms developed on the inoculated maize seeds and seedlings were compared with those of control run experiment. The germination rate of seeds of different cultivars of different districts was determined by keeping all records on paper/computer. The data were formulated in matrix form and proceeded for statistical analysis and evaluation according to the ISTA rules (1996).

## Biochemical Analysis

### *Carbohydrate estimation*

Carbohydrate content: The amount of total soluble sugars was estimated using anthrone method (Hedge *et al.*, 1962). The principle of the method is anthronereaction is the basis of a rapid and convenient method for the determination of hexoses, aldopentoses, and hexuronic acids either free orpresent in polysaccharides. Carbohydrates are dehydrated by conc. $H_2SO_4$ to form furfural. Furfural condenses with anthrone to form a blue-green coloured complex which is measured colorimetrically at 630 nm. Following reagentswere used to estimate carbohydrate i.e. 80% ethanol, anthrone reagent and standard glucose One hundred milligram powdered sample was grounded in pestle and mortar with 5mL 80% ethanol. The homogenates were placedin centrifuge plastic tubes and then centrifuged at 10,000 rpm for 10 minutes. The supernatant solution was collected in tubes and used to estimate soluble sugar. Then the volume was made up to 20 mL with 80% ethanol. From the above 20 mL solution 0.5 mL aliquots of sample were taken. The standards were also prepared by taking 0.2, 0.4, 0.6, 0.8,and 1-mL of the standard glucose solution. '0'served at blank. The volumes were made up to 2mL in all the tubes including the sample tubesand the blank by adding distilled water. Then anthrone reagent was added. After heating in boiling water bath, the samples were cooled and the green to dark green coloured solution was read at 630 nm against blank. The standard curve was drawn byplotting the concentration of the standard and the amount of soluble sugars presented in the sample tube was calculated from the graph.

### *Protein estimation*

The methodology established by Lowery *et al.* (1951)was employed to ascertain the protein content of maize grain stored samples. The procedure involves incubating the samples with a freshly prepared protein reagent consisting of 2% Na2CO3 in 0.5 M NaOH, 1% NA-K tartrate, and 1% $CuSO_4$ in a ratio of 100:1:1. Subsequently, the reaction mixture wastreated with 1 N Folin's reagent to initiate the development of a blue colour. The measurement of absorbance at 660 nm was conducted, followed by comparing absorbance values to a standard plot generated by utilizing varying concentrations 0.2,0.4,0.6,0.8,1-mL of BSA (Bovine Serum Albumin). The protein concentration was expressed as in mg/ml. Finally, the concentration of protein was calculated using the formula:

*The concentration of protein = Standard O.D x Observed O.D.*

### *Germination test*

A sample of 30 grains was taken from the representative samples. The samples were placed separately in moist germination paper covered with another sheet of the paper then rolled and kept in polythene bags to prevent moisture loss. There were three replications for each sample, a complete randomized design. The observation of seed germination was recorded separately for each sample after 6 days. The effect on germination of maize worked out by following the formula of Thippeswamy & Lokesh (1977).

*Mean germination loss (%) = No. of germinated seeds/ No. of seeds kept for germination x 100*

## Data Analysis

Fungal pathogen count data were performed at least three times and results were specified using standard error and mean by using Microsoft Excel.

Table 1: Fungal isolated through Agar plate method from unsterilized and sterilized maize grains collected from Bilaspur district.

| Fungal species identified | Maize grains collected from different Tahsils | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Masturi | | Kota | | Ratanpur | | Sipat | | Sakri | | Bilaspur | |
| | UG | SG | UG | SG | UG | SG | UG | SG | UG | SG | UG | SG |
| *A. flavus* | - | + | + | + | + | + | + | + | + | + | + | + |
| *A. niger* | - | + | + | - | + | + | + | + | + | + | + | + |
| *A. fumigatus* | - | + | - | + | - | - | + | - | + | + | - | + |
| *A. tereus* | - | + | + | - | - | - | - | - | + | - | + | - |
| *A. ochraceous* | + | - | - | - | + | + | - | + | + | + | - | - |
| *C. cladosporioides* | - | - | - | - | - | - | - | - | + | + | - | - |
| *F. oxysporum* | + | + | + | - | - | - | + | + | - | - | - | + |
| *F. moniliforme* | + | + | + | - | - | - | + | - | - | + | - | - |
| *A. alternata* | - | - | - | - | - | + | + | + | - | - | + | + |
| *P. chrysogenum* | + | + | + | + | - | + | - | + | - | - | - | + |
| *R. stolonifera* | - | - | - | - | - | + | + | - | + | - | - | + |
| *T. viride* | - | - | + | - | - | - | - | - | - | + | - | - |
| *Mucor spp.* | + | + | - | - | - | + | - | - | - | + | - | - |
| *C. lunata* | + | - | - | - | - | + | + | - | - | - | + | + |
| *N. sphaerica* | - | - | - | - | + | - | - | - | - | - | + | - |

+ : presence of fungi, – : the absence of fungi, UG : Unsterilized grains, SG : Sterilized grains

Table 2: Fungal isolated through Blotter paper method from unsterilized and sterilized maize grains collected from Bilaspur district

| Fungal species identified | Maize grains collected from different Tahsils | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Masturi | | Kota | | Ratanpur | | Sipat | | Sakri | | Bilaspur | |
| | UG | SG | UG | SG | UG | SG | UG | SG | UG | SG | UG | SG |
| *A. flavus* | - | - | + | + | + | + | + | + | + | + | + | + |
| *A. niger* | - | + | + | + | + | - | + | + | + | + | + | - |
| *A. fumigatus* | - | + | - | + | - | - | + | - | + | + | - | + |
| *A. tereus* | - | + | + | - | - | - | - | - | + | - | + | - |
| *A. ochraceous* | + | - | - | - | + | + | - | + | + | + | - | - |
| *F. oxysporum* | + | - | - | - | + | + | + | - | + | + | + | + |
| *F. moniliforme* | - | - | - | - | - | - | + | + | - | - | - | + |
| *R. stolonifera* | + | + | + | - | - | - | + | - | - | + | - | - |
| *Mucor spp.* | - | - | - | - | - | + | + | + | - | - | + | + |
| *A. alternata* | + | + | + | + | - | + | - | + | - | - | - | + |
| *C. cladosporioides* | - | - | - | - | - | + | + | - | + | - | - | + |
| *P. chrysogenum* | - | - | + | - | - | - | - | - | - | + | - | - |
| *S. chrysospermum* | + | + | - | - | - | - | - | - | - | + | - | - |
| *Helminthosporium spp.* | + | - | + | - | - | + | + | - | - | - | + | + |

+ : presence of fungi, – : the absence of fungi, UG : Unsterilized grains, SG : Sterilized grains

## RESULTS AND DISCUSSION

### Identification of Fungi

The present investigation revealed the district related to fungal identifications in the seeds of maize are mentioned in Table 1 from different tahsils of Bilaspur district. All together 15 species and 12 species were found respectively of fungi were isolated from maize seeds through Agar plate technique. These include *A. flavus, Aspergillus niger, Aspergillus terreus, Aspergillus fumigatus, Aspergillus ochraceus, Fusarium oxysporum, Fusarium moniliforme, Cladosporium cladosporioides, Penicillium chrysogenum, Alternaria spp., Rhizopus stolonifera, Trichoderma viride, Mucor spp., Curvularialunata, Nigrosporasphaerica*. The most common fungi isolated from stored grainswere *A. flavus, A. niger, F. oxysporum* (Fig. 2) in Bilaspur District of intermediate agro-ecology. The results indicated that in all five districts, these fungi were consistently recorded throughout the storage period. In all of the studied districts, the presence of *A. fumigatus, C. cladosporioides, A. terreus* and *Penicillium* spp. were inconsistent with the increase in the storage days. During the present study, the species *P. chrysogenum* and *A. terreus* were detected in a district found under intermediate agro-ecology and they were detected only from maize samples stored in Bilaspur area.

It is also evident from the data in Table 2, through Blotter paper method by both unsterilized and sterilized method, 14 species and 10 species were found respectively i.e. *A. flavus, A. niger, A. terreus, A. fumigatus, A. ochraceus, F. oxysporum, F. moniliforme, R. stolonifera, Mucor spp., Alternaria alternate, C. cladosporiodes., Penicillium sp., Helminthosporium spp., Sepedoniumchryso spermum* were observed. It also revealedthat *A. flavus, A. niger,* and *F. oxysporum* were the most frequent species of fungi recorded over the storage periods from 90%, 51%, 44% of the seed samples respectively, whereas *A. Fumigatus* and *C. Cladosporioides* were detected from 3.6 and 15% samples, respectively. Both *A. terreus* and *Penicillium* species were the most dominant species of fungi being detected only from 0.5% of the samples and sterile white mycelium was detected from 10% ofseed samples.

Janardhana *et al.* (1999); reported Mycotoxin contamination in maize grains grown in the state Karnataka which revealed that there are 25 fungal species belonging to 14 genera isolated from Agar plate technique and Blotter paper technique like *Aspergillus flavus, A. flavuscolumnaris, A. niger, A. nidulans, A. sydowi, A. fumigatus, A. terreus, A. falvusoryzae, A. viscolor, A. candidius, Rhizopus spp., Penicillium spp., Curvularialunata, Fusarium spp., Alternaria alternate, Botrydiplodia theobromae, C. cladosporoides, Cumninghamella spp., Drechslera turcica, D. teteramera, F. moniliforme, F. oxysporum, F. solani, Mucor spp., Nigrosporaoryzae, Verticillum spp.* Another investigation revealed the presence often fungal speciesin agar plate method of study viz., *Aspergillus candidus, A. flavus, A. niger, A. terreus, A. ruber, Rhizopus spp., Penicillium spp., Curvularia spp., Fusarium spp., Alternaria spp.* in the maize seeds during storage (Bhattacharya & Raha, 2002).

Mohana & Raveesha (2007) investigated anti-fungal evaluation of plant extracts against storage fungi and suggested that there are 10 fungal species were isolated and identified *i.e. F. moniliforme, A. niger, A. flavus, Fusarium graminearum, F. oxysporum, F. proliferatum, F. solani, A. candidus, A. columnaris, Pencillium griseofulvum* out of these *Aspergillus* (62-100%), *Penicillium* (28-66%) and *Fusraium* (44-95%) were found to be most dominant fungi (Kulkarni *et al.*, 2010); isolated Five fungal species *i.e. Alternaria alternate, F.oxysporum, Helminthosporium tetramera, Pencillium notatum, Rhizoctonia solani* etc. and were identified from Agar plate technique and 11 species from Blotter paper technique from 15 varities ofmaize seeds like *A. flavus, A. niger, A. fumigatus, A .ustus, F. oxysporum, F. moniliforme, H. tetramera, Mucor globus, R. solani, Rhizopus stolonifer, Pencillium spp.* (Nagaraja *et al.*, 2016). An investigation of atotal 53 maize samples revealed that *Fusarium* species like *F. graminearum* (79.24%), *F. verticillioides* (77.35%) were found to be dominantand *F. acominatum* (3.77%) was found to be leastinmaize grains.

In Pakistan, Niaz *et al.*, 2009 stated that 56 species belonging to 23 genera of fungi were isolated and identified through agar plate method and blotter paper method. Dominant species like *A. flavus, A. niger, A. wentii, Penicillium spp* were reported through agar plate method while in Deep freezing method *Dreschslera spp., Fusarium spp., Penicillium spp.* were isolated.

Yilma *et al.* (2019) stated that 9 species were isolated from maize grains like *A.flavus, A. paraticus, F. verticilloides, P. notatum, P. verrucosum, F. proliferaum, F. gramminearum, A. noiger, Trichoderma spp.* in the West Showa and East Wallega Zones part of Ethiopia. Dudoiu *et al.* (2016) mentioned that Mycoflora associated with maize rains during storage period of 60, 90, and 120 days in Romania through Agar plate technique by sterilized and both Unsterilized methods revealed that *Alternaria, Cladosporium, Penicillium, Mucor, Cephalosporium, Aureobasidium, T. viride.* While in Blotter paper methods fungal species identified as *Fusarium spp.* Like *F. moniliforme, F. tricinctum, F. graminearum, Cephalosporium spp., Aspergillus spp., Alternaria tennuissima, Cladosporium herbarum, T. viride, Nigrosporasphaerica, Sclerotiniasclerotiorum, Mucor*

Table 3: Occurrence frequency of fungi isolated through Agar plate method ( Unsterilized and Sterilized) of stored maize in Bilaspur district

| Fungal species | Occurrence frequency in Bilaspur district | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Masturi | | Kota | | Ratanpur | | Sipat | | Sakri | | Bilaspur | |
| | UG | SG | UG | SG | UG | SG | UG | SG | UG | SG | UG | SG |
| *A. flavus* | 23.33 | 11.23 | 40.1 | 28.38 | 2.5 | 1.23 | 45.6 | 15.28 | 27.2 | 16.2 | 69.8 | 30.2 |
| *A. niger* | 62.21 | 21.25 | 65.0 | 34.9 | 32.7 | 12.4 | 39.1 | 12.6 | 43.9 | 10 | 57.7 | 28.90 |
| *A. fumigatus* | 8.1 | 2.4 | 11.0 | 10.2 | 2.6 | 1.6 | 3.4 | 2.7 | 6.8 | 4.9 | 7.1 | 6.23 |
| *A. tereus* | 7.9 | 3.4 | 8.1 | 6.41 | 5.8 | 2.13 | 4.2 | 2.6 | 6.3 | 2.8 | 7.6 | 5.1 |
| *A. ochraceous* | 4.0 | - | 6.6 | 3.21 | 4.2 | 2.1 | 8 | 4.3 | 12.9 | 8.2 | 4.8 | 2.9 |
| *C. cladosporioides* | - | - | 0.3 | - | - | - | 5.2 | 3.21 | - | - | 7.3 | - |
| *F. oxysporum* | 23.2 | 13.25 | 11.6 | 10.7 | 7.9 | 6.8 | 10.2 | 8.34 | 12.6 | 4.78 | 21.8 | 11.67 |
| *F. moniliform* | 21.1 | 14.1 | 10 | 9.67 | 3.4 | 1.2 | - | - | 11.2 | - | 1.9 | - |
| *C. cladosporiodes* | - | - | 0.3 | - | 1.9 | 1.23 | - | 0.4 | 5.5 | 3.21 | - | - |
| *P. chrysogenum* | 19.3 | 11.8 | 18.6 | 11.4 | 29.1 | 21.8 | 8 | 4.67 | 14.3 | 11.34 | 3.6 | 2.9 |
| *A. alternate* | 0.93 | - | - | - | 0.3 | 1.2 | 4.5 | 2.7 | - | 4.9 | - | 2.0 |
| *T. virdie* | 2.7 | 1.3 | 5 | 2.89 | 2.3 | 2.1 | 2.1 | 1.6 | - | 1.54 | 1.9 | - |
| *Mucor spp.* | 8.3 | 6.03 | 2.6 | - | 1.8 | - | 4.2 | 3.6 | 1.1 | - | 5.5 | 2.5 |
| *N. sphaerica* | - | - | - | - | 3.9 | 1.9 | - | 0.54 | - | 0.67 | - | - |
| *R. stolonifera* | 0.4 | - | 5.4 | 2.17 | 0.6 | - | 3.0 | 1.5 | 0.6 | - | 22.8 | 21.09 |

Table 4: Occurrence frequency of fungi isolated through Blotter Paper method (unsterilized and sterilized) of stored maize in Bilaspur district

| Fungal species | Occurrence frequency in Bilaspur district | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Masturi | | Kota | | Ratanpur | | Sipat | | Sakri | | Bilaspur | |
| | UG | SG | UG | SG | UG | SG | UG | SG | UG | SG | UG | SG |
| *A. flavus* | 24.3 | 5.4 | 44.3 | 15.7 | 1.5 | 0.5 | 34.6 | 20 | 17.2 | 10.2 | 49.8 | 26.2 |
| *A. niger* | 37.4 | 21.5 | 45.0 | 23.6 | 22.7 | 10.2 | 19.1 | 10.1 | 23.9 | 21 | 47.7 | 11.1 |
| *A. fumigatus* | 5.2 | 2.0 | 11.0 | 6.2 | 2.6 | 1.1 | 3.4 | - | 6.8 | 4.2 | 7.1 | 5.2 |
| *A. tereus* | - | - | 7.5 | 2.5 | 5.8 | 2.8 | 4.2 | 2.2 | 6.3 | 3.1 | 7.6 | 5 |
| *A. ochraceous* | 4.8 | 1.4 | 6.4 | 2.1 | - | - | 8.6 | 6.1 | 6.9 | 5.6 | 2.8 | 2.8 |
| *C. cladosporioides* | - | - | 0.3 | | - | - | 5.2 | - | - | - | - | - |
| *F. oxysporum* | 13.2 | 11.1 | 5.6 | 5.1 | 4.9 | - | 7.2 | 3.1 | 8.6 | 4.1 | 13.8 | - |
| *F. moniliforme* | 19.1 | 3.1 | 10 | - | 3.4 | 1.2 | - | - | 12.2 | - | 1.9 | - |
| *S. chrysospermum* | - | - | 0.3 | - | 1.9 | - | - | 0.3 | 1.5 | - | - | - |
| *P. chrysogenum* | 14.3 | 11.6 | 6.6 | 6.3 | 18.1 | 16.2 | 7.3 | 2.3 | 12.3 | 7.1 | 2.6 | 0.4 |
| *A. alternate* | 0.9 | - | - | - | 0.2 | - | 6.5 | - | - | - | - | 1.2 |
| *T. virdie* | 2.7 | 1.5 | 5 | 3.1 | 2.3 | 2.1 | 2.1 | - | - | - | 1.9 | 1 |
| *Mucor spp.* | 8.2 | 6.1 | 2.6 | 1.7 | 1.8 | 0.3 | 4.2 | 2.1 | 1.1 | - | 5.5 | - |
| *R. stolonifera* | - | - | - | - | 3.6 | 1.2 | - | 4.0 | - | 2.7 | - | - |
| *Helminthosporium spp.* | 0.4 | - | 5.4 | 1.3 | 0.6 | 0.2 | 3.0 | - | 0.6 | - | 2.8 | 1.6 |

*pussilum, A. niger, Alternaria spp., Penciliium frequentans, Periconia spp., Aureobasidium spp., Stemphillium botryosum, R. stolonifera*. In the state-Kebbi, Nigeria Aminu *et al.* (2021) suggested that there are 8 fungal species were isolated from maize grains like *A. niger, A. fumigatus, A. terreus, Fusraium spp, Cephalosporium spp., A. flavus, Penicillium spp.*

Goko *et al.* (2021) reported the identification and characterization of seed-borne fungal pathogens associated with maize grains revealed that there were seven fungal species isolated through Agar plate method like *F. moniliforme, R. stolonifera, Penciliium citrinum, Aspergillus flavus, A. paraticus, A.niger, A.tamarii*. According to Sadia *et al.* (2021), prevalence of fungi associated with storage seeds of several maize varieties. In Dhaka, Bangladesh revealed that there were 7 fungal species isolated i.e. *A. flavus, A. fumigatus Fresenius, A. niger van Teighem, Curvularialunata, F. verticilloides, Pencillium italicum, Rhizopus stolonifer.* Beyene *et al.* (2024) investigated by observinginsect pests' diseases in stored maize grains and suggested that, through Agar plate technique and Blotter paper method *Aspergillus flavus, A. paraticus, A. niger* were found to be dominant and additionally insect species like *Sitophilus zeamays*, *S. cerealella, S.oryzae, Triboleum castanewum, T.confusum, R. dominica* species were infected the maize grains during the storage in West Hararghe, Ethiopia.

## Protein Estimation

The protein levels varied from 10.82 to 13.59%, according to the results shown in Table 5. The outcome is comparable to that of Orhun *et al.* (2013), who found that the protein content varied from 8 to 11%. Additionally, this conclusion is consistent with other research conducted by several experts, which found that the lowest protein percentage in maize kernels was found to be 5.7% and the greatest protein percentage was 15.8% (Singh *et al.*, 2004). But in our findings, Protein percent was found to be reduced i.e. 7.04% due to the effect of *A. flavus* as compared to control. In our findings Protein content was maximum reduced in the case of *A. flavus* (7.04%) and *A.niger* (8.37%), as compared to the control set. It could be due to the deterioration caused by *A. flavus,* similarly, in the case of *A.niger*, it was 46.45% and least reduction was seen in the case of *A. ochraceous* (9.42%) and *F. oxysporum* (8.40%).

## Carbohydrate Estimation

The carbohydrate levels ranged from 63.03 to 69.36%, according to the data. This conclusion is consistent with the findings of Iken *et al.* (2002), who found that the range of carbohydrate amounts was 72-73 percent. The variation in the quantity of carbohydrate may be due to the application of nitrogen. If nitrogen was applied more than the amount of carbohydrates, the amount of carbohydrates dropped, and if nitrogen was applied less than the amount of carbohydrates, the amount of carbohydrates rose (Singh *et al.*, 2004). In our findings Carbohydrate content was maximum reduced approximately 30% i.e. 40.23% as compared to the control set. It could be due to the deterioration caused by *A. flavus,* similarly, in case of *A.niger*, it was 46.45% and least reduction was seen in the case of *A. ochraceous* (49.58%) and *F. oxysporum* (48.23%).

## CONCLUSIONS

Fungi could cause about 50-80% damage of maize during storage if conditions are favourable for their development. During the storage period, maize grains develop a series of microorganism. The fungal organisms were identified as the species of *viz., Aspergillus, Alternaria*, *Cladosporium, Penicillium, Cephalosporium, Mucor, R. stolonifera, T. viride,* and *Fusarium.*

Several fungi observed in the present study are known to produce mycotoxins which are harmful to human health mycotoxinscan cause severe damage to liver, kidney, and nervous system of human beings even in low dosages.The present study revealed *Aspergillus* species like *A. flavus* and *A. niger* were found to be the most predominant species in stored maize grain. This concurs with the

Table 5: Deterioration in fresh Maize grains caused by dominant fungi in respect to weight loss, germination and biochemical contents

| | Weight loss in seeds (g) | | Percent germination of seeds(%) | | Protein contents (%) | | Carbohydrate content (%) | |
|---|---|---|---|---|---|---|---|---|
| Fungal species | Control | Treatment | Control | Treatment | Control | Treatment | Control | Treatment |
| *A. flavus* | No loss | 0.29 | 91.1 | 50.3 | 13.58 | 7.04 | 69.36 | 40.23 |
| *A. niger* | - | 0.37 | 84.56 | 43.0 | 13.58 | 8.37 | 69.36 | 46.45 |
| *F. oxysporum* | - | 0.16 | 82.4 | 62.4 | 13.58 | 8.40 | 69.36 | 48.23 |
| *A. ochraceous* | - | 0.11 | 72.3 | 37.6 | 13.58 | 9.42 | 69.36 | 49.58 |

literature that *Aspergillus* moulds can be attributed to factors such as warmth and high relative humidity with low temperatures, which may result in improper drying of the maize as well as high temperatures with drier conditions, which predisposes maize to moulds in storage. There are weather conditions favouring the fungal establishment in maize, hence threatening its safety during storage as well. Various studies also support this study's findings that seed-borne fungal pathogens can infect maize preharvest and increase mycotoxin levels under different storage facilities if conditions are poorly managed.

Preventive measures, such as fast drying of maize for medium and long-term storage in hygiene-maintained, without the presence of insects and microorganisms, and proper regulation of grains moisture content, could significantly reduce the fungi contamination of maize grains. Further work is in progress in our laboratory to mitigate this issue through botanical fumigants.

## Conflict of Interest

The authors declare that there are no conflicts of interest.

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