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Understanding the seed health status of stored paddy seed of archeologically important places of Assam, India

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Abstract
Seeds can be considered as the most efficient means for pathogen dispersal. Seeds can harbor a wide range of pathogens especially fungi, that can drastically reduce the germinability of seed. Pathogens can move along with the seed from one place to another. Thereby spreading the disease to newer areas. Seed-borne pathogens can also act as human or animal pathogens by producing mycotoxins. Seed storage is the most widely accepted method for preserving genetic resources. Temperature, humidity, and moisture present in the seed influence the build-up of inoculum potential of fungi during storage. The farming community is less aware of seed-pathogen relations. The farmers of Majuli-the world's biggest river island and Charaideo- the pyramid of Assam maintained the traditional cultivars for a long back. To investigate the seed health status of the stored seed of paddy by the farmers, the present investigation is carried out to collect the stored seed and evaluate the diversity of mycoflora responsible for seed deterioration by standard blotter and agar test method. Seed germination and moisture content of the seed in storage was also evaluated. Seed discoloration was found to be major problem in those traditional varieties associated with seed mycoflora *viz*., *Fusarium* spp., *Drechslera oryzae*, *Curvularia lunata*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* spp. etc.

Keywords: Seed borne pathogen, blotter test, seed health, mycoflora

1. Introduction
Study on seed health is an important aspect as seed is the basic planting material of all agricultural activities. Seeds should be free from seed-borne or seed-associated pathogens to earn a good harvest. Seeds are also considered a highly effective means for carrying the pathogen over long distances (Fraedrich 2009) [2]. Various examples are available for virus-transmitted diseases that were responsible for epidemics. Maize lethal necrosis, Wheat Yellow Dwarf disease, Wheat streak mosaic diseases, Rice tungro diseases, Potato necrotic ring spot diseases, banana bunchy top diseases, and *Citrus tristeza* disease have been proven as devastating diseases (Jones 2021) [3]. It is estimated that 30% of seed-borne diseases can be managed by using disease-free seed.

Many pathogens can survive in the seed for years together. Seeds are generally stored in cool and dry conditions. This condition is also favourable for the survival of many pathogenic structures inside the seed, although survivability will be decreased with increased storage duration (Brodal and Asdal, 2021) [4]. Seed is the reservoir of many essential nutrients like carbohydrate, protein and minerals which is utilized by pathogenic seed-associated fungi or bacteria and continue to survive by drawing the nutrients from seed only and become pathogenic to the growing seedling from that infected seed. However, seed also contains defensive chemicals like phenolic compound *viz*., polyphenol oxidase, peroxidases and oxalate oxidase and lectin to prevent pathogen infection. For successful pathogenicity, pathogenic microbes specially necrotrophs counteract such barriers than obligate parasites of seed (Nallathambi *et al*., 2021) [1]. Viral pathogens may enter to the seed through male or female gametophytes during flowering and systemically transmit from seed to seedling and to adult plants. Fungi that harbour the seed may be divided into two categories *viz*., field and storage fungi, Field fungi are those that invade the seed in field itself when the seed is attached the its mother plant or during its maturity. *Alternaria*, *Fusarium*, and *Drechslera* are the most commonly occurring field fungi of seed. Seeds require almost 80-90% relative humidity for growth of field fungi. While storage fungi Aspergillus and Penicillium can build up their inoculum potential in storage conditions with seed moisture at equilibrium with relative

humidity 65 to 90%. They are mostly saprophytes or weak pathogen. The major contribution of these fungi is to cause discoloration and production of mycotoxin that ultimately reduce germination. During storage, if moisture content is high it will cause spontaneous heating that will increase the respiratory activity and ultimately lead to drastic change in the quality of the seed (Martin *et al*., 2022) [5].

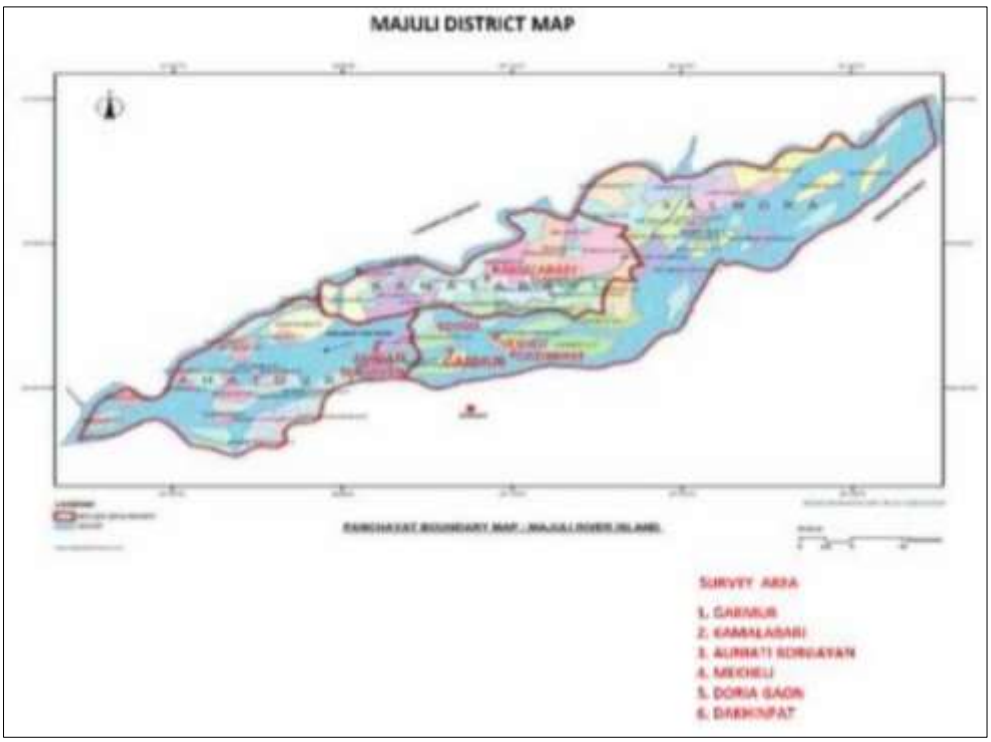
The world's largest river island- Majuli, is indeed a spiritual home place of ancient Hindu monasteries or Satras, laid by the great Assamese saints Srimanta Sankardeva and Madhavdeva that have been preserved as well as nurtured traditional vibrant Assamese culture for the generations. Chariadeo- The pyramid of Assam- the burial grounds of Ahom kings and Queens, was the original capital of the Ahom Kingdom, established in the Brahmaputra valley of Assam in 1228 and ruled for about 600 years. Both Majuli and Charaideo districts is preserving indigenous local rice cultivars that have been cultivated since long back and used to store the harvested seed with traditionally made structures. The study on the seed health of those indigenous local cultivars is an important aspect as the seed is the basic planting material of all agricultural activities.

2. Methodology

2.1 Collection of seed sample

All together hundred three farmers-saved paddy seeds were collected directly from the seed storage structures of five (5) different divisions, comprising ten villages of Majuli district of Assam and four (4) different blocks of Charaideo district of Assam during 2021-22 (Plate 2a and 2b). Farmers saved paddy seeds were bought directly to the seed pathology laboratory, AAU, Jorhat in sterilized poly bags by asserting sample numbers to each of the samples and kept at ambient temperature in the laboratory for further analysis.

A.



B.

Plate 1 (a and b): District map of Charaideo and Majuli of Assam India. Red dots indicates the surveyed areas

2.2 Estimation of seed moisture content
Seed moisture has a significant role in the development of seed mycoflora. Seed moisture content was estimated for the collected seed samples with digital moisture meter and illustrated in Fig 1.

2.3 Estimation of seed germination
Seed germination test was carried out for each of the samples separately by rolled paper towel method as per the ISTA protocol. Briefly, the minimum sample size for this experiment was 400 seeds per sample that were counted with a digital automatic seed-counting machine. A hundred seeds were placed in each of the sterilized germination papers. The paper was pre-soaked in sterile distilled water and 100 numbers of seeds were placed over the paper with sterilized forceps. Wrap the paper with the rubber band and then incubated in the germination chamber. Two counts were recorded for the germination test. Germination percent was calculated by the following formula:

Seed germination (%) = Number of total seeds germinated/total number of seeds kept for germination×100

2.4 Detection of seed mycoflora
2.4.1 Detection of bunt diseases
The seeds were tested for infection of bunt disease by soaking the seed in 0.2 per cent NaOH solution for 24 hours for the detection of bunt disease. After 24 hours the solution was decanted and the swollen seeds were spread over a blotter to remove excess moisture. The seeds are visually examined for brown, dull or shiny black discoloration.

A B

Plate 2 (a and b): Survey and collection of sample

2.4.2. Detection by blotter method
The pathogen associated with the seed discoloration was recorded by blotter method and agar plate method. The blotter test was carried out as per the ISTA protocol. Briefly, three layers of blotter sheets (water holding capacity of 40 cc) were placed in a sterile 90 mm petri plate. Moisten the three layers of filter paper with sterile distilled water and drain out the excess water. Seeds were surface sterilized with 1% NaOCl and subsequently washed three times with sterile distilled water. Twenty-five (25) numbers of seeds were placed aseptically in each petri plate and kept under the incubator at 22±1 °C with an alternate period of day and night for 6 days of incubation. The filter paper was moistened every three days intervals with sterile distilled water so that the paper would not dry out during the experiment. Prevalence of fungal species and their morphological studies have been done based on nature of conidiophore, the arrangement of the conidiophore and the shape and size of the conidia. The fungi appearing on the seeds were isolated, purifed and their single spore cultures were maintained on Czapek's Dox agar medium.

2.4.3. Detection by agar plate method
For the agar plate method, Czapek's Dox Agar medium with the following compositions was used for the whole experimentation. The ingredients used in the media were (Gms/lit) (Sucrose 30.00; Sodium nitrate 2.00; Dipotassium phosphate 1.00; Magnesium sulphate 0.50; Potassium chloride 0.50; Ferrous sulphate 0.01 and Agar 15.0). The medium was sterilized in an autoclave for 15 lb/square inch pressure at 121 °C for 20 minutes. After the media cooled down, 10 mg of streptomycin was thoroughly mixed into the media to prevent bacterial contamination, and poured into the sterilized 90 mm petri dish in the laminar airflow chamber and allowed to solidify. With the help of a sterilized needle, 10 seeds from each sample were placed aseptically at an equidistance position placing one in the center. The plates were kept in BOD incubator keeping an inverted position at temperature 28±2 °C for 7 days. Three replicates for each variety were maintained.
The percent frequency of occurrence of pathogenic mycoflora was calculated by the following formula:
Frequency (%) = Number of plates in which individual fungal species occurred/ total number of plates studied × 100 (Kumar *et al*., 2023) [6].

3. Result and discussion
The farmers use conventional agricultural practices to store the paddy seeds in the *Dully* (Bamboo mud plastered structure) (Plate 3a) HDPE bag, jute bag, (Plate 3b) wooden and semi-concrete *Bharal* (plate 3c) and *Muthi bharal* (Plate 3d) after sun drying. It was observed that 75% of the farmers directly stored their harvested paddy in *Jute bag*s and rest were stored in different storage structures as mentioned above.
Seed moisture content is one of the most important factors in determining seed quality (Agarwal *et al*., 1994) [7]. Pathogen buildup is directly related to seed moisture content. Higher seed moisture favors pathogen build up range of seed moisture was recorded that varies from 10.00 to 16.20% for

the sample collected from Charaideo and 11.95-18.20% for the samples collected from Majuli district. Accordingly, germination also varies with seed moisture content. Seed germinability was found to be less in those seeds having seed moisture content Much higher or lower than Indian Minimum Seed Certification standard (IMSCS). It was recorded that 29.41% and 23.30% of total seed collected from Charaideo and Majuli district showed germination below IMSCS in rolled paper towel method (Plate 4). Seeds having high seed moisture content and germination percent below IMSCS were taken into consideration for further study for association of seed mycoflora in blotter as well as agar plate method. The associated pathogen and the frequency of occurrence of different pathogens from the stored seed were recorded (Table 1 and Fig 1). Fungal mycelia of the infected seed was observed to be spread to the blotter paper in few pathogens after 6 days of incubation. Conidia as well as mycelial character of the fungus were also observed by Zeiss Axioskopt2 microscopy (Carl Zeiss Microscopy Company, Germany) Fungal species observed in blotter test as well as agar plate method are described below.

Plate 3 (a): Dully

Plate 3 (b): HDPE and Jute bag

Plate 3 (c): Wooden and semi-concrete Bharal

Plate 3 (d): Muthi bharal

Plate 4: Rolled paper towel method for seed germination

3.1 *Bipolaris oryzae* (Breda de Haan) Shoem., syn. *Drechslera oryzae*, syn. *Helminthosporium oryzae* Breda de Haan

Light grey aerial mycelia were produced on the seed coat and covering whole part of the seed. Appeared as fluffy growth. Conidiophore with conidia was observed under high power (Fig.) as septed, curved and tapering at the end or crescent shaped, light brown, widest in the middle and tapering to the rounded ends (Plate 5a). Light grey arial mycelia covering the whole seed coat, occasionally spread to the blotters (Plate 5b). The infected seed if germinated produced rotted seedling (Plate 5c, d).

Plate 5 (a): Conidiophore and conidia of *Drechslera oryzae* (40X)

Plate 5(b): Mycelia covering the seed coa

Plate 5 (c and d): Rotting of germinated seedling

3.2 *Fusarium* spp.

Fusarium spp. is one of the important seed-borne pathogens that cause bakane diseases of rice. The pathogen was observed as white cottony growth in the infected paddy seed (Plate 6a). Both macro and microconidia were observed. Microconidia were single septed and macroconidia were 4-5 septed (Plate 6b).

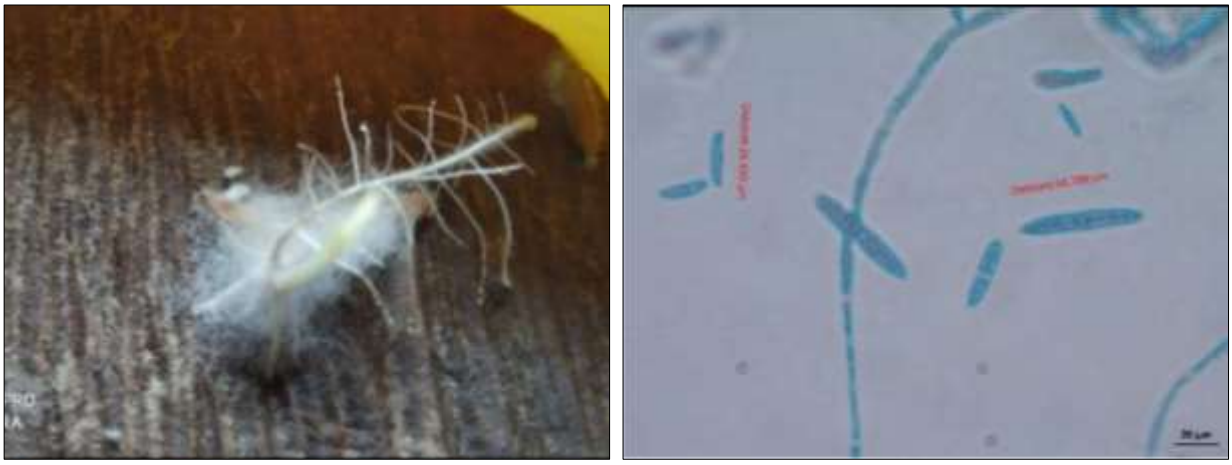


Plate 6 (a and b): White cottony growth of infected seeds. Macro and micro conidia of *Fusarium* spp

3.3 *Curvularia* spp.

Curvularia is one of the important seed-borne pathogens responsible for seed discoloration and early seedling blight and seed rot. The growth of the pathogen in PDA was fast, with brownish-black to black in both forward and reverse. Conidiophores are erect, septate, geniculate. Conidia were ellipsoidal, curved, rounded at the ends, pale brown, septed (Plate 7).

Plate 7: *Curvularia* spp from infected seed

3.4 *Nigrospora* spp.

The species is very fast growing with septed, hyaline hyphae. Conidiophores are hyaline or slightly pigmented, bears single conidia at apex. Conidia are black, unicellular, solitary, with thin equatorial germ slit (Plate 8).

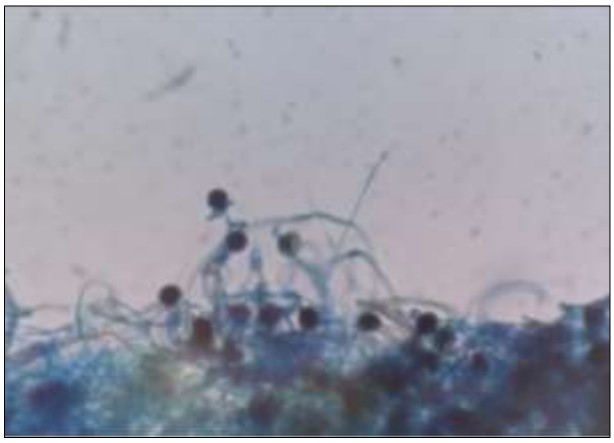


Plate 8: *Nigrospora* spp

3.5 *Penicillium* spp.

Penicillium is the most widely occurring pathogen with branch-like conidiophore arranging conidia in conidial apparatus known as Penicillus, derived from the latin word penicillium. Meaning small brush, composed of stalk that bears a tufts of conidiogenous cell known as metulae. Conidia are globose, smooth wall produce in chain, in large quantities and hyaline under microscope (Plate 9 and b). Greenish mycelial mat of the pathogen was developed covering the infected seed.

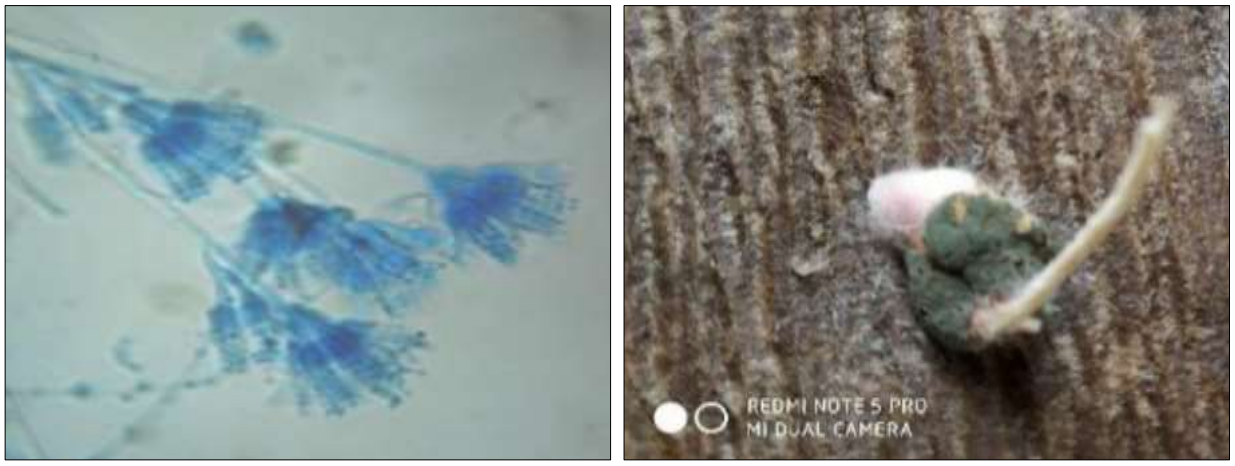


Plate 9 (a and b): *Penicillium* spp. (40 X) and mycelial mat covering the entire seed

3.6 *Aspergillus niger*

The genus *Aspergillus* produced a special brush-shaped structure mostly found in air, soil, spoiling food, clothes etc. It is the most common species of *Aspergillus* found mainly in food spoilage. Conidiophores are erect, produce vesicles bearing metulae (Plate 10 a and b)

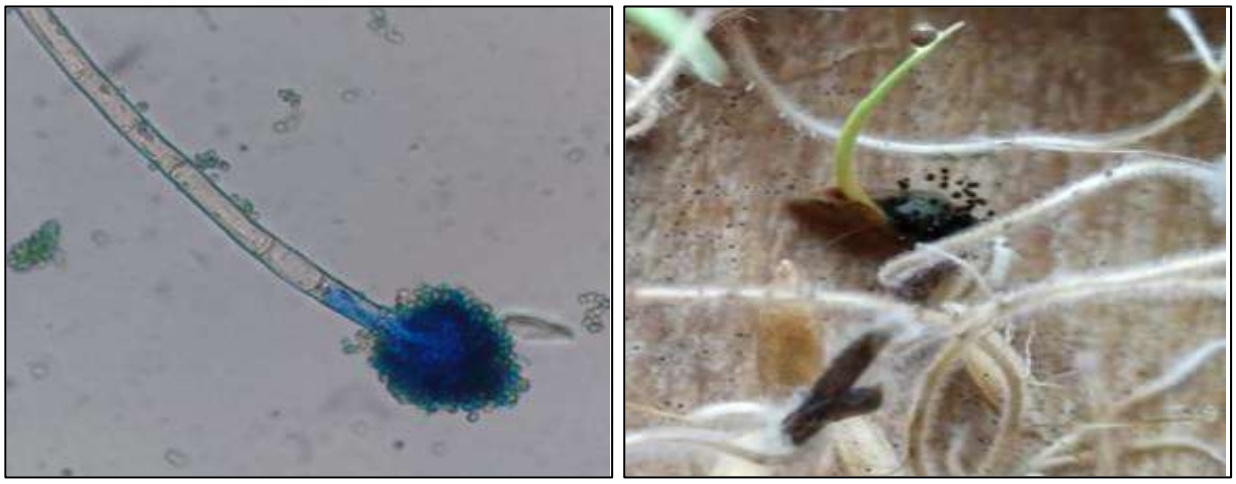


Plate 10 (a and b) Conidia and conidiophores of *Aspergillus niger* and infected see

Plate 11: *Aspergillus flavus*

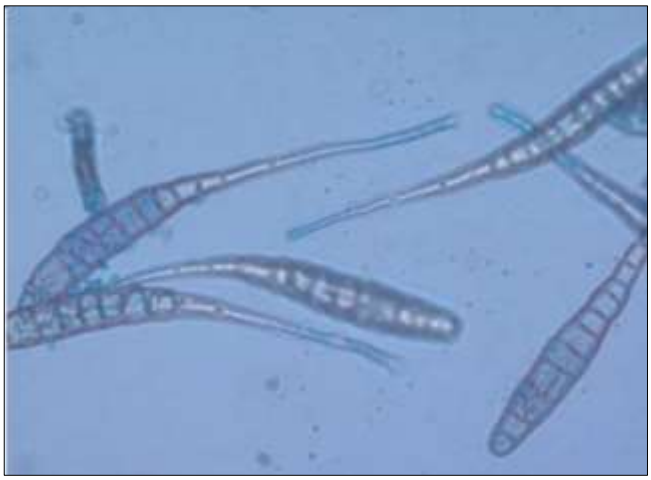


Plate 12: *Alternaria* spp (40X)

3.7 *Aspergillus flavus*

Aspergillus flavus is the most notorious and known to produce aflatoxin in different food stuffs. The colony colour is greenish yellow in PDA.

The asexual state is dominant in nature and mycelium produces stout, erect conidiophores abundantly. The terminal swollen part is known as vesicle on which bottle-shaped structures known as phialides are produced. As the conidiogenous cells reach maturity, they produce chains of conidia at their tips. The shape of the conidia is globose (Aspergillus 11).

3.8 *Alternaria* spp.

Alternaria is one of the most widely occurring pathogen. In seed it may occur as saprophyte also. The pathogen produces conidia that occur as sub hyaline and later turn as straw to brown in colour. Conidia with beak (Plate 12).

3.9 *Rhizopus* spp.

Rhizopus belongs to the family Rhizophoraceae with the order Mucorales. The fungal body is characterized by mycelia producing three types of hyphae, stolon, rhizoids and sporangiophores. The black sporangia produce at the tip of the sporangiophores containing numerous smooth-walled, rounded and non-motile conidia (Plate 13)

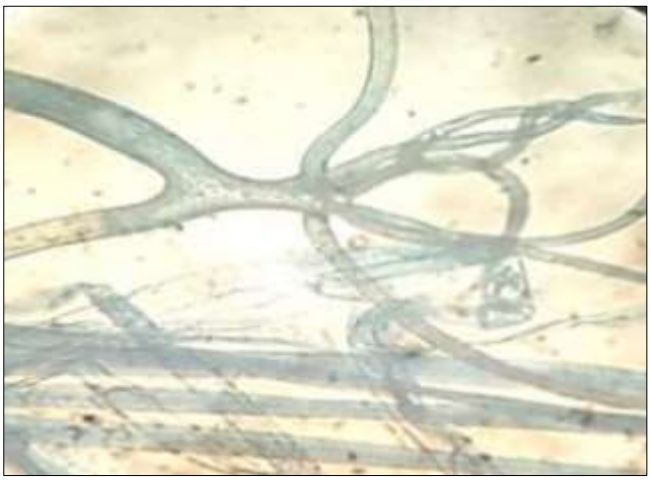


Plate 13: *Rhizopus* with rhizoids (40X)

It was observed that all of the above-mentioned pathogens are responsible for seed discoloration causing severe reduction in germination and vigour of the seedling. Severely discolored samples recorded seed germination below the Indian Minimum Seed Certification Standard (IMSCS). NaOH test for detection of bunt pathogen was found negative for all the treatments.

Table 1: Number of fungal species recorded from different places of Majuli and Charaideo district of Assam, India

Sl. No.	Pathogen species	Variety							
		Aghuni bora, Charaideo	Bat bao	Bora dhan	Jahingia	Malbhog	Manuhar sali	Miyasali	Konjoha
1	*Alternaria*	-	-	-	+	-	-	-	-
2	*Aspergillus flavus*	+	+	-	+	-	-	+	+
3	*Aspergillus niger*	+	-	+	+	-	-	+	+
4	*Curvularia lunata*	-	-	-	-	+	-	-	-
5	*Fusarium* spp.	-	+	-	+	-	-	-	-
6	*Penicillium*	+	-	-	-	+	-	+	-
7	*Nigrospora oryzae*	-	-	+	-	-	-	-	-
8	*Drechslera oryzae*	+	-	+	+	-	+	+	+
9	*Rhizopus* spp.	-	-	-	-	-	+	-	-

Number of Fungal species recorded from Majuli and Chraideo district of Assam. +: Presence of fungal species; −: Absence of fungal species

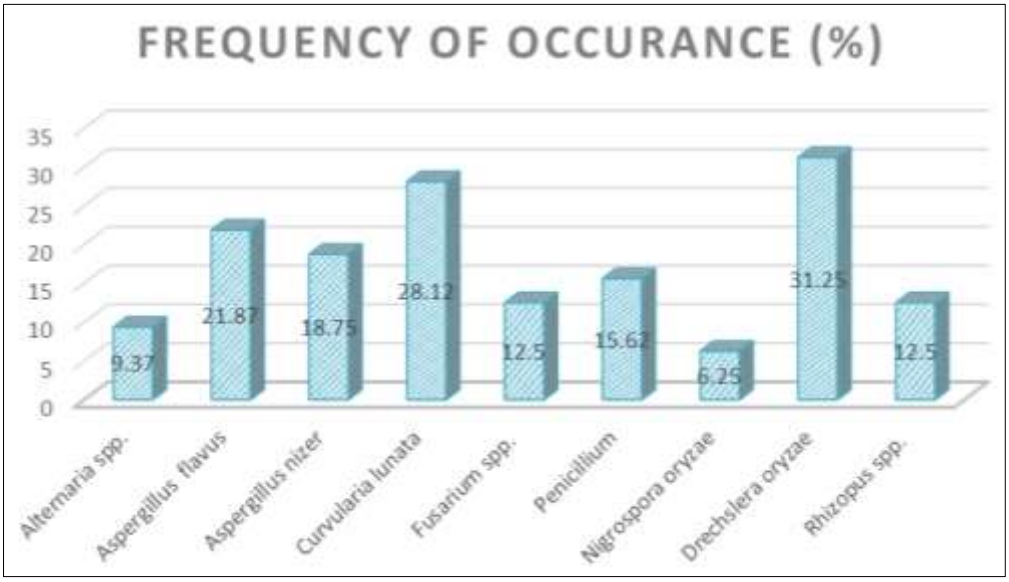


Fig 1: The relative occurrence and percentage frequency of fungal isolates

It was observed that though the farmers of Majuli and Charaideo- the two archeologically important places of Assam cultivated the traditional rice cultivars for years together, the seeds that were stored for next year's sowing were found to be higher moisture content that leads to germination below IMSCS. Also, such types of seeds were found to be infected with many seed-borne pathogens that drastically reduced the seed quality as well. The farmers of these region are need to be aware of the proper seed storage facility that keep optimum moisture content during storage**.**

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