Analysis of seed borne mycoflora of some economically important crop plants grown in Barpeta district of Assam, India

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In the present study, pre-dominant fungi isolated by both Blotter and Nutrient Agar Plate methods from the different seeds of black gram, mung bean and chilli were Alternaria alternata, Aspergillus clavatus, A.fumigatus, A.flavus, A.niger, Cladosporium cladosporoides, Cladosporium herbarum, Fusarium oxysporum, Mucor hiemalis, Penicillium citrinum, Rhizopus stolonifer and Trichoderma viride. The other less occurring fungi isolated from the sampled seeds were Chaetomium sp., Colletotrichum capsici, Nigrospora sp., Helminthosporium sp., Phoma sp. and Torula sp. Results revealed that in terms of frequency of occurrence in the two methods of isolation the number of fungi is higher in the Nutrient Agar Plate than the Blotter method. Culture filtrates of some predominantly occurring fungi viz-Aspergillus flavus, A. niger, Curvularia lunata, Fusarium oxysporum, Mucor hiemalis, Rhizopus stolonifer and Trichoderma viride were taken. The maximum inhibition of germination of seeds was recorded when the seeds were treated with Aspergillus niger and minimum inhibition was recorded with culture filtrate of Fusarium oxysporum. The percentage of seed germination decreased with the increase of seed storage period. It is clear that seeds like black gram, mung and chilli are affected by different types of fungi which reduce their quality and percentage of seed germination.

Keywords: Black gram, chilli, culture filtrates, Mung, seed-borne mycoflora,

INTRODUCTION

Seed can be defined as a mature fertilized ovule that possesses an embryo, stored material in the form of cotyledons or endosperm and a protective seed coat. Seeds contain both pathogenic and saprophytic micro-organisms, both externally and internally and reduce their quality and they affect seed germination and resulting in the production of abnormal seedlings (Amza, 2018). It is generally known that the soil microflora and rhizosphere microflora exhibit a closed relationship with seed mycoflora. Pathogen associated with seeds externally and internally and these organisms become active under favourable conditions lead to the drastic yield reduction.

Black gram (*Vigna mungo* L.) belongs to the family Fabaceae, is grown as one of the important pulse

crops.There are a number of micro-organisms responsible for disease of the seed and low germination and reduced yield. Several workers have studied the mycoflora associated with black gram seed (Agarwal et al. 2011, Biswal et al. 2019 and Kandhare, 2020). Mung or green gram (Vigna radiata L.) is one of the most important leguminous crops of the arid and semi-arid tropics. Bakr and Rahaman (2001) reported a number of mycoflora such as Alternaria sp., Fusarium oxysporum, Aspergillus flavus, A. niger etc. are associated with the seeds of mung bean. Devamani et al. (2017) studied eighteen different samples of mung bean and isolated twelve fungi belonging to ten genera .Chilli is an important vegetable crop planted in almost all parts of tropical and subtropical regions of the world. Chilli is affected by diseases like root and collar rot and the diseasecausing organism is *Phytophthora capsici* (Than et al. 2008). Chilli is susceptible to the several diseases like root and collar rot, die-back and fruit

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rot, powdery mildew (Sitara and Hasan, 2011). The mycoflora associated with chilli seeds were extensively studied by Alam *et al.* (2014).

It has been reported that seed germination is greatly influenced by seed borne fungi which are known to produce some toxic metabolites. Some seed borne fungal metabolites possess both inhibitory and stimulatory effects on the germination of different kinds of seeds. Jalander and Gachande (2012) worked on the effect of culture filtrates of rhizosphere fungi of pigeonpea viz. Aspergillus flavus, A.niger and A.nidulans on seed germination and seedling growth of some cereals and pulses and they reported that the secondary metabolites of three species of Aspergillus inhibited the seed germination and root-shoot length of all the selected cereals and pulses. They found the metabolites of A.niger to be more effective than those of A.flavus and A.nidulans. Kushwaha (2021) has reported that seed fungi reduced germinability of mung bean seeds and also affected seedling health with mortality percentage 8.6 to 37.6%. The farmers of the district produce a large number of crops plant specially black gram, mung and chilli and huge amounts of these crops are exported to different parts of the state. But the information about the seed borne mycoflora of black gram, mung and chilli of Barpeta district is insufficiently available. Therefore, the present study was carried out to analyses seed mycoflora, effect of culture filtrates on seed germination and seedling growth of black gram, mung and chilli.

MATERIALS AND METHODS

Seeds of Black gram (*Vigna mungo* L.), Green Gram or Mung (*Vigna radiata* L.) and Chilli (*Capsicum annuum* L.) were collected from the local markets at regular intervals and were stored in different containers for further studies. The experimental work was done in the Microbiology laboratory, Post Graduate Department of Botany, Madhab Choudhury College, Barpeta, Assam in 2022. The different methods used were as follows-

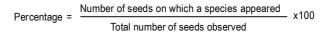
Blotter method

The standard Blotter method, as described by International Seed Testing Association was used

for isolation of seed borne fungi. Three layers of blotters (No. 1 Whatman filter paper) were soaked in sterile distilled water and placed in sterile glass Petridishes (9 cm dia). Ten seeds from each sample were plated per petridish, then incubated them at 27±1°C in an incubator for seven days and every third day sterile distilled water was sprayed aseptically on the Petriplates to keep them moist. After seven days of incubation, the colonies per plate were counted and then microscopic slides were prepared and examined under microscope for identification of the isolated fungi.

Nutrient Agar Plate method

For the study of seed mycoflora, nutrient agar plate method as described by Muskett (1948) was used. In this experiment, 10 seeds from each samples collected plated, surface and non-surface sterilized; then plated in Petriplates containing approximately 20 ml Potato Dextrose Agar medium supplemented with antibiotic Amoxycillin Trihydrate (Novamox; 0.0025gm/100ml media) to inhibit the bacterial growth. The surface sterilization was done with 0.1% HgCl, the seeds were treated with HgCl, for one minute then washed with sterilized distilled water for 3 times. All the petri dishes containing the seeds were incubated at 27±1°C for seven days. During the incubation period, the cultured plates were observed constantly and recorded growth characters of fungal colonies carried by the cultured seeds. The growing fungi were pure cultured on suitable Potato Dextrose Agar slants for further examination. In each case, percentage of fungal incidence was recorded as per the methods of Dutta and Roy (1987). The percentage incidence of individual fungus was calculated by the following formula -



Fungi were identified on the basis of morphological and reproductive characters and were confirmed as per keys and methods proposed by Gilman (1957); Funder (1968); Barnett and Hunter (1972). Some of the fungi were identified upto species level and some were upto generic level.

Germination test

30 seeds of each sample were selected randomly,

surface sterilized with 0.1% mercuric chloride (HgCl₂) and placed in Petridishes (10 seeds in each) on single layer of Whatman filter paper No.1 and kept them at room temperature (Buriro *et al.*2011).Distilled water was sprayed whenever necessary to keep the seeds moistened. The shoot and root lengths were recorded after 8-10 days. The germination percentage of seeds was calculated by the following formula-

Moisture content

Seed moisture percentage was determined by crushing the seeds to powder form and weighing fresh, then subjecting for 48 hrs at 60°C temperature and again reweighing it. Seed moisture percentage was represented on the original weight basis as described by Srivastava and Sareen (1972).

Effects of fungal culture filtrates on seed germination and seedling growth of sampled seeds

Some predominant fungi of selected seeds taken for this experiment were *Aspergillus flavus*, *Aspergillus niger, Curvularia lunata, Mucor hiemalis, Rhizopus stolonifer, Fusarium oxysporum* and *Trichoderma viride*. Each fungus was cultured separately in 250ml conical flask filled with 100ml Czapeck's dox liquid medium and kept them in an incubator at 27±1°C for 15 days. With the help of Whatman No.1 filter paper the content of the conical flasks were filtered after 15 days. Then their effects on the seed germination and seedling growth were observed.

The experiment was carried out according to the method described by Vijayan and Rehill (1990). First of all, sampled seeds were surface sterilized with 0.1% mercuric chloride (HgCl₂) and washed with sterile distilled water. Ten seeds were placed in each sterilized petri dish. The culture filtrates were poured into the Petridishes containing the individual seeds. The seeds were placed in petridish containing moist blotter paper and the control seeds were treated with sterile distilled

water. The effects of fungal cultures on seeds germination were recorded on the 4th day and seedling growth were recorded on the 7th day of inoculation. Seedling growth was recorded by taking the average of 10 seedlings.

RESULTS AND DISCUSSION

The results (Tables 1 & 2; Fig.1) showed that a total of 13 different fungal species belonging to 10 genera were isolated in different number and percentage by Blotter method of which Aspergillus niger showed the highest percentage of occurrence (15.6% in SS and 14.6% in NSS seed), while 19 different fungal species belonging to 14 genera were isolated by Nutrient Agar Plate method where Aspergillus niger showed the highest percentage of occurrence (15.3% in SS seed) and Aspergillus flavus (13.5% in NSS seed) of blackgram seeds. In the present study, the predominating fungi that consistently occurred and proliferated in the blackgram seeds were Alternaria alternata, Aspergillus clavatus, A.fumigatus, A.flavus, A.niger, Chaetomium sp., Cladosporium cladosporoides, Curvularia lunata, Fusarium oxysporum, Mucor hiemalis, Penicillium citrinum, Rhizopus stolonifer and Trichoderma viride. The present findings are similar with the findings of Agarwal et al. (2011); Biswal et al. (2019); Debbarma et al. (2019) and Kandhare (2020). Agarwal et al. (2011) isolated seed-borne fungi from black gram such as Alternaria alternata, Aspergillus niger, A. flavus, Chaetomium sp., Fusarium oxysporum, Penicillium citrinum and Rhizopus nigricans. Biswal et al. (2019) identified six fungal species associated with blackgram seeds which were Curvularia lunata, Fusarium pallidoroseum. Macrophomina phaseolina, Rhizopus sp., Aspergillus flavus, A. niger and Penicillium sp. Debbarma et al. (2019) isolated a total of five pre-dominant seed -borne fungi viz., Aspergillus sp., Penicillium sp., Rhizopus sp., Rhizoctonia sp. and Trichoderma sp., from five pulse seeds in Nagaland. Kandhare (2020) isolated A. flavus, A. fumigatus, A. niger, Drechsleratetramera, Fusarium moniliforme and Rhizopus stolonifer from seeds of Green gram and Black gram.

The results (Tables 3 &4; Fig. 2) showed that a total of 14 different fungal species belonging to 12 genera were isolated in different number and percentage by Blotter method of which *Aspergillus*

| Fungal types isolated | Surface sterilized (SS) | Non-Surface sterilized | | ntage of rrence |
|---------------------------|-------------------------|---------------------------|------|--------------------|
| | | (NSS) | SS | NSS |
| Alternaria alternata | 2 | 4 | 6.25 | 6.25 |
| Aspergillus clavatus | - | 3 | - | 4.68 |
| A. flavus | 2 | 5 | 6.25 | 7.81 |
| A. niger | 5 | 9 | 15.6 | 14.06 |
| Aspergillus sp. | 3 | 4 | 9.37 | 6.25 |
| Chaetomium sp. | - | 3 | - | 4.68 |
| Cladosporium cladosporoid | les 1 | 3 | 3.12 | 4.68 |
| Curvularia lunata | 2 | 4 | 6.25 | 6.25 |
| Fusarium oxysporum | 3 | 5 | 9.37 | 7.81 |
| Mucor hiemalis | 4 | 6 | 12.5 | 9.37 |
| Penicillium citrinum | - | 2 | - | 3.12 |
| Rhizopus stolonifer | 3 | 6 | 9.37 | 9.37 |
| Trichoderma viride | 2 | 4 | 6.25 | 6.25 |
| Unclassified group | 3 | 3 | 9.37 | 4.68 |
| Dark sterile mycelium | 2 | 3 | 6.25 | 4.68 |
| Total | 32 | 64 | | |

| Table 1: Number and percentage occurrence of some | nportant seed borne fungi of bl | ck gram isolated by Blotter method |
|---|---------------------------------|------------------------------------|
|---|---------------------------------|------------------------------------|

niger showed the highest percentage of occurrence (13.3% in SS and 12.3% in NSS seed), while 19 different fungal types belonging to 14 genera were isolated by Nutrient Agar Plate methodwhere Aspergillus niger showed the highest percentage of occurrence (12.28% in SS seed and 15.51% in NSS seed) in mung bean seeds. The pre-dominating fungi that consistently occurred and proliferated in the seed samples isolated were Alternaria alternata, Aspergillus flavus, A.niger ,Chaetomium sp., Cladosporium cladosporoides, Curvularia lunata, Fusarium oxysporum, Helminthosporium sp., Mucor hiemalis, Penicillium citrinum, Phoma sp., Rhizopus stolonifer, Torula sp. and Trichoderma viride. Similar observations were also made by Devamani et al. (2017) and Kushwaha (2021). Devamani et al. (2017) reported 12 fungi belonging to 10 genera associated with mung and the isolated fungi were Aspergillus niger, A. flavus, A. candidus, Alternaria alternata, Penicillium notatum, Rhizopus stolonifer, Cladosporium sp., Fusarium oxysporum, Mucor sp., Curvularia lunata, Chaetomium globosum and Macrophomina phaseolina. Kushwaha (2021) isolated 15 fungal species belonging to 11 genera from mung bean seeds and the isolated predominant fungal types were *Alternaria alternata*, *Aspergillus flavus*, *A.niger*, *A. fumigatus*, *Colletotrichum capsici*and *Rhizopus stolonifer*.

The results (Tables 5 & 6; Fig. 3) showed that a total of 16 different fungal species belonging to 13 genera were isolated in different number and percentage by Blotter method where Aspergillus niger showed the highest percentage of occurrence (13.88% in SS and 11.11% in NSS seed), while 16 different fungal species belonging to 13 genera were isolated by Nutrient Agar Plate method of which Aspergillus niger showed the highest percentage of occurrence (14.28% in SS seed and 11.70% in NSS seed)in chilli seeds. The predominating fungi that consistently occurred and proliferated in chilli seeds were Alternaria alternata, Aspergillus fumigatus, A. flavus, A.niger, Chaetomium sp., Colletotrichum capsici, Curvularia lunata, Fusarium oxysporum. Helminthosporium sp., Mucor hiemalis, Penicillium citrinum, Phoma sp., Rhizopus stolonifer, Torula sp. and Trichoderma viride. Similar observations were also made by Alam et al. (2014) who reported that

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| Fungal type isolated | Surface sterilized(SS) | Non-Surface sterilized(NSS) | Percentage of occurrence | | |
|----------------------------|---------------------------|--------------------------------|--------------------------|------|--|
| | | | SS | NSS | |
| Alternaria alternata | 2 | 5 | 3.84 | 4.50 | |
| Aspergillus clavatus | - | 2 | - | 1.80 | |
| A.fumigatus | 2 | 4 | 3.84 | 3.60 | |
| A. flavus | 5 | 15 | 9.61 | 13.5 | |
| A. niger | 8 | 11 | 15.3 | 9.90 | |
| Aspergillus sp. | - | 2 | - | 1.80 | |
| Chaetomium sp. | - | 3 | - | 2.70 | |
| Cladosporium cladosporoide | es 2 | 4 | 3.84 | 3.60 | |
| Curvularia lunata | - | 6 | - | 5.40 | |
| Fusarium oxysporum | 3 | 10 | 5.76 | 9 | |
| F.moniliformae | 2 | 5 | 3.84 | 4.50 | |
| Helminthosporium sp. | - | 3 | - | 2.70 | |
| Mucor hiemalis | 5 | 8 | 9.61 | 7.20 | |
| Nigrospora sp. | - | 4 | - | 3.60 | |
| Penicilliumcitrinum | 3 | 6 | 5.76 | 5.40 | |
| Phoma sp. | 2 | - | 3.84 | - | |
| Rhizopusstolonifer | 5 | 9 | 9.61 | 8.10 | |
| Torulasp. | 1 | - | 1.92 | - | |
| Trichoderma viride | 3 | 6 | 5.76 | 5.40 | |
| Darksterilemycelium | 4 | 5 | 7.69 | 4.50 | |
| Unclassified | 3 | 3 | 5.76 | 2.70 | |
| Total | 52 | 111 | | | |

Table 2: Number and percentage occurrence of some important seed borne fungi of black gram isolated by Nutrient Agar Plate method

Colletotrichum capsici, Curvularia lunata, Aspergillus flavus, Fusarium moniliforme and Rhizopus stolonifer were found to be associated with untreated seeds of chilli which reduced the germination of the seeds. The above results revealed that in terms of their frequency of occurrence in the two methods of isolation the number of fungi is higher in the Nutrient Agar Plate than the Blotter method.

The results (Table 7) showed that the effects of tested fungal culture filtrates on the germinability of the sampled seeds black gram, mung and chilli were highly pronounced. As compared to the control, some of the fungal culture filtrates showed inhibitory effect and the others showed promotive effect on the germination of seeds. The culture

filtrates treated with the sampled seeds, after the treatment (Table 7), it was observed on the 4thday that 70%,10%, 90%, 100%, 90%, 50% & 60% of Black gram; 60%, 0%, 90%, 60%,80%,70% & 70% of Mung seeds had germinated when treated with the culture filtrates of *Aspergillus flavus*, *A.niger*, *Curvularia lunata*, *Fusarium oxysporum*, *Mucor hiemalis*, *Rhizopus stolonifer* and *Trichoderma viride* respectively as compared to the control. The germinability of chilli seeds was 0% it means treatment of these culture filtrates with the seeds of chilli inhibited their germination completely.

It was observed that there exists a varying degree of reduction in germinability of seeds soaked in the culture filtrates. The loss of germination of the seeds was found higher in the culture filtrate of *Aspergillus niger* as compared to the other fungi.

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| Fungal types isolated | Surface sterilized(SS) | Non-Surface sterilized (NSS) | Percent | |
|---------------------------|---------------------------|---------------------------------|---------|------|
| | | | SS | NSS |
| Alternaria alternata | 1 | 3 | 3.33 | 4.61 |
| Aspergillus flavus | 2 | 6 | 6.66 | 9.23 |
| A. niger | 4 | 8 | 13.3 | 12.3 |
| Aspergillus sp. | - | 4 | - | 6.15 |
| Chaetomium sp. | - | 3 | - | 4.61 |
| Cladosporium | 2 | 4 | 6.66 | 6.15 |
| cladosporoides | | | | |
| Curvularia lunata | 2 | 4 | 6.66 | 6.15 |
| Fusarium oxysporum | 3 | 5 | 10 | 7.69 |
| Helminthosporium sp. | - | 2 | - | 3.07 |
| Mucor hiemalis | 3 | 6 | 10 | 9.23 |
| Phoma sp. | 2 | - | 6.66 | - |
| Rhizopus stolonifer | 3 | 7 | 10 | 10.7 |
| <i>Torula</i> sp. | - | 1 | - | 1.53 |
| Trichoderma viride | 3 | 4 | 10 | 6.15 |
| Unclassified group | 2 | 3 | 6.66 | 4.61 |
| Dark sterile mycelium | 3 | 5 | 10 | 7.69 |
| Total | 30 | 65 | | |
| | | | | |

Table 3: Number and percentage occurrence of some important seed borne fungi of mung isolated by Blotter method

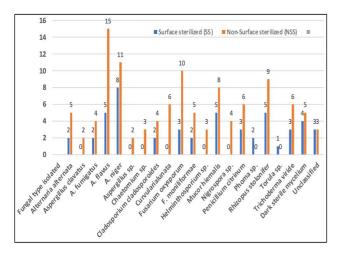


Fig. 1 : Number of occurrence of some seed borne fungi of Blackgram evaluated through Nutrient Agar Plate Method

The fungi which showed promotive effects on the germination of black gram and mung were *Curvularia lunata, Fusarium oxysporum, Mucor hiemalis, Rhizopus stolonifer* and *Trichoderma viride.*

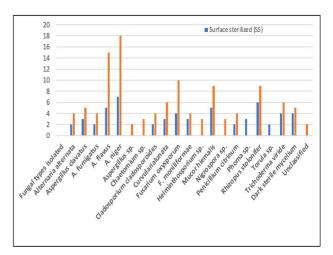


Fig. 2 : Number of occurrence of some seed borne fungi of Mung evaluated through Nutrient Agar Plate Method

The average seedling heights (Table 7) of black gram were 0.56cm, 0.1cm, 2.65cm, 1.85 cm,3.15cm, 1.75cm and 1.05cm when treating with the culture filtrates of *Aspergillus flavus, A.niger*,

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| Fungal types isolated | Surface Non-Surface sterilized(SS) sterilized(NS | | Percent occur | | |
|-----------------------------|---|-----|------------------|-------|--|
| | | | SS | NSS | |
| Alternaria alternata | 2 | 4 | 3.50 | 3.44 | |
| Aspergillus clavatus | 3 | 5 | 5.26 | 4.31 | |
| A.fumigatus | 2 | 4 | 3.50 | 3.44 | |
| A. flavus | 5 | 15 | 8.77 | 12.93 | |
| A. niger | 7 | 18 | 12.28 | 15.51 | |
| Aspergillus sp. | - | 2 | - | 1.72 | |
| Chaetomium sp. | - | 3 | - | 2.58 | |
| Cladosporium cladosporoides | 2 | 4 | 3.50 | 3.44 | |
| Curvularia lunata | 3 | 6 | 5.26 | 5.17 | |
| Fusarium oxysporum | 4 | 10 | 7.01 | 8.62 | |
| F.moniliformae | 3 | 4 | 5.26 | 3.44 | |
| Helminthosporium sp. | - | 3 | - | 2.58 | |
| Mucor hiemalis | 5 | 9 | 8.77 | 7.75 | |
| <i>Nigrospora</i> sp. | - | 3 | - | 2.58 | |
| Penicillium citrinum | 2 | 4 | 3.50 | 3.44 | |
| Phoma sp. | 3 | - | 5.26 | - | |
| Rhizopus stolonifer | 6 | 9 | 10.52 | 7.75 | |
| Torula sp. | 2 | - | 3.50 | - | |
| Trichoderma viride | 4 | 6 | 7.01 | 5.17 | |
| Dark sterile mycelium | 4 | 5 | 7.01 | 4.31 | |
| Unclassified | - | 2 | - | 1.72 | |
| Total | 57 | 116 | | | |

Table 4 : Number and percentage occurrence of some important seed borne fungi of mung isolated by Nutrient Agar Plate method

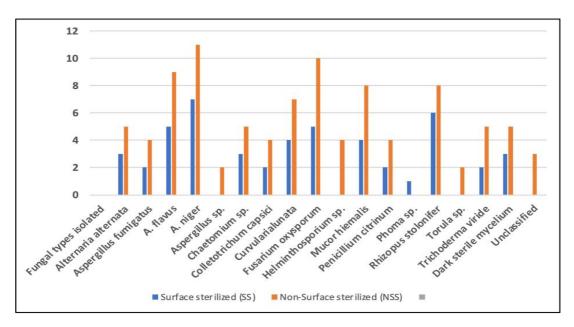


Fig. 3 : Number of occurrence of some seed borne fungi of Mung evaluated through Nutrient Agar Plate Method

| Fungal types isolated | Surface sterilized(SS) | Non-Surface sterilized(NSS) | Percen occur | tage of rence |
|------------------------|---------------------------|--------------------------------|-----------------|------------------|
| | | | SS | NSS |
| Alternaria alternata | 2 | 3 | 5.55 | 4.76 |
| Aspergillus fumigatus | 1 | 2 | 2.77 | 3.17 |
| A. flavus | 4 | 5 | 11.11 | 7.93 |
| A. niger | 5 | 7 | 13.88 | 11.11 |
| Aspergillus sp. | 2 | 3 | 5.55 | 4.76 |
| Chaetomium sp. | - | 2 | - | 3.17 |
| Colletotrichum capsici | - | 3 | - | 4.76 |
| Curvulari alunata | 2 | 5 | 5.55 | 7.93 |
| Fusarium oxysporum | 3 | 4 | 8.33 | 6.34 |
| Helminthosporium sp. | - | 2 | - | 3.17 |
| Mucor hiemalis | 3 | 6 | 8.33 | 9.52 |
| Penicillium citrinum | 1 | 4 | 2.77 | 6.34 |
| Phoma sp. | 2 | - | 5.55 | - |
| Rhizopus stolonifer | 4 | 6 | 11.11 | 9.52 |
| <i>Torula</i> sp. | - | 2 | - | 3.17 |
| Trichoderma viride | 2 | 4 | 5.55 | 6.34 |
| Unclassified group | 2 | - | 5.55 | - |
| Dark sterile mycelium | 3 | 5 | 8.33 | 7.93 |
| Total | 36 | 63 | | |

Table 5: Number and percentage occurrence of some important seed borne fungi of chilli isolated by Blotter method

Curvularia lunata, Fusarium oxysporum, Mucorhiemalis, Rhizopus stolonifer and Trichoderma viride respectively. The average seedling heights (Table 8) of mung bean were 0.7cm, 6.45cm, 2.2cm, 2.6cm, 1.5cm and 0.09cm when treating with the culture filtrates of Aspergillus flavus, Curvularia lunata, Fusarium oxysporum, Mucor hiemalis, Rhizopus stolonifer and Trichoderma viride respectively. The seeds of chilli remain the same after treating with the fungal culture filtrates. The culture filtrates of these above-mentioned fungi inhibited the seed germination as well as subsequent seedling growth (Table 7). Similar observations were also obtained by Kandhare (2020) who found that green gram seed when treated with fungal culture filtrates, the seed germination (%) was ranged from 30.0 to 80.0%, compared to 100.0 % in the control, and A. niger showed highest effective. The shoot lengths were in the ranges of 1.5-4.0 cm in infected seeds,

compared to 6.3cm in the control, where *A. niger* also highly reduced the shoot length. The seed germination(%) of seeds borne fungi treated black gram seeds was ranged from 30.0 to 89.0%, compared to 90% in the control, where *A. niger* also the highest effective.

In the present study ,the germination of sampled seeds was inhibited by the culture filtrate of *Aspergillus niger* due to production of oxalic acid and some toxic metabolites. According to Rettinassababady *et al.* (2000) that some fungi can produce certain growth promoting substances and hormones. In the present investigation a higher promotive effect was found on the seeds of black gram and mung when they were treated with the culture filtrates of *Fusarium oxysporum, Mucor hiemalis, Rhizopus stolonifer and Trichoderma viride.* It is clear that chilli seeds are also influenced by different types of seed-borne fungi which reduce

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| Fungal types isolated | Surface sterilized(S | Non-Surface sterilized(NSS) | Percentage of occurrence | |
|------------------------|-------------------------|--------------------------------|--------------------------|-------|
| | S) | | SS | NSS |
| Alternaria alternata | 3 | 5 | 6.12 | 5.31 |
| Aspergillus fumigatus | 2 | 4 | 4.08 | 4.25 |
| A. flavus | 5 | 9 | 10.20 | 9.57 |
| A. niger | 7 | 11 | 14.28 | 11.70 |
| Aspergillus sp. | - | 2 | - | 2.12 |
| Chaetomium sp. | 3 | 5 | 6.12 | 5.31 |
| Colletotrichum capsici | 2 | 4 | 4.08 | 4.25 |
| Curvularia lunata | 4 | 7 | 8.16 | 7.44 |
| Fusarium oxysporum | 5 | 10 | 10.20 | 10.63 |
| Helminthosporium sp. | - | 4 | - | 4.25 |
| Mucor hiemalis | 4 | 8 | 8.16 | 8.51 |
| Penicillium citrinum | 2 | 4 | 4.08 | 4.25 |
| Phoma sp. | 1 | - | 2.04 | - |
| Rhizopus stolonifer | 6 | 8 | 12.24 | 8.51 |
| <i>Torula</i> sp. | - | 2 | - | 2.12 |
| Trichoderma viride | 2 | 5 | 4.08 | 5.31 |
| Dark sterile mycelium | 3 | 5 | 6.12 | 5.31 |
| Unclassified | - | 3 | - | 3.19 |
| Total | 49 | 94 | | |

Table 6: Number and percentage occurrence of some important seed borne fungi of chilli isolated by Nutrient Agar Plate method

Table 7: Effect of fungal culture filtrates on the germination percentage of seeds and seedling growth (in cm) of black gram (*Vigna mungo* L.), mung (*Vigna radiata* L.) and chilli (*Capsicum annuum* L.) on the 7th days of Treatments

| Treatments | Crop Seed | | | | | |
|---------------------|-----------|------------|------|------|-----|-----|
| rreatments | Blac | Black Gram | | Mung | | lli |
| | | | | | | |
| Control | 100% | 7.2 | 100% | 9.25 | 30% | 0.4 |
| Aspergillus flavus | 70% | 0.56 | 60% | 0.7 | 0% | 0 |
| Aspergillus niger | 10% | 0.1 | 0% | 0% | 0% | 0 |
| Curvularia lunata | 90% | 2.65 | 90% | 6.45 | 0% | 0 |
| Fusarium oxysporum | 100% | 1.85 | 60% | 2.2 | 0% | 0 |
| Mucor hiemalis | 90% | 3.15 | 80% | 2.6 | 0% | 0 |
| Rhizopus stolonifer | 50% | 1.75 | 70% | 1.5 | 0% | 0 |
| Trichoderma viride | 60% | 1.05 | 70% | 0.9 | 0% | 0 |

their seed quality, germination and develop abnormal seedlings.

CONCLUSION

Seeds of black gram, mung and chilli harbors a heterogeneous group of fungi which can reduce the quality of seeds, germination percentage and inhibit seedling growth by producing some toxic chemicals. Seed should be stored in clean dry containers by maintaining the seed moisture content. Seeds should be treated with fungicides (Chemical/ Bio) before sowing.

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REFERENCES

- Agarwal, T., Abhiniti, M., Trivedi, P.C. 2011. Fungi associated with chickpea, lentil and black gram seeds of Rajasthan. *Int. J.Pharma and Bio Sci.* **2**:478-483.
- Alam, M.Z., Hamim, I., Ali, M.A., Ashrafuzzaman, M. 2014. Effect of seed treatment on seedling health of Chilli. J. Environ. Sci. Natural Resour.7:177-181.
- Amza, J. 2018.Seed Borne Fungi; Food Spoilage, Negative Impact and Their Management: A Review. Food Sc. Qua. Management. 81: 70-79.
- Bakr, M. A., Rahaman, M. L. 2001. Research findings of BARI on seed borne diseases of pulses. *Proc.National Workshop on Seed Pathol.* 45-52.
- Barnett, H.L., Hunter, B.B. 1972. Identification of species of *Curvularia* on rice seed. *Proc. Intl. Seed Test. Asso.*35: 99-119.
- Biswal, J., Rana singh, N.; Sahu, K.C.; Moharana, R.L., Behera, S. 2019."Seed Health of Farmers" Saved Black Gram (Vigna mungo L. Heppper) Seedsin Western Undulated Zones of

Odisha. Int.J.Curr.Microbiol.App.Sci. 8:2738-2742.

- Buriro, M., Oad, F. C., Keerio, M. I., Tunio, S., Gandahi, A. W., Hassan, S. W., Oad, S.M., 2011. Wheat seed germination under the influence of Temperature Regimes. *Sarhad J. Agric*. 27:539-543.
- Debbarma, A. Banik, S. Ao, N. T. 2019. Seed-borne mycoflora of pulses in Nagaland. J. Mycopathol. Res.57: 179-184.
- Devamani, B.D., Saifulla, M., Jayappa Sab, J. 2017. Prevalence of Seed mycoflora of Mung bean (*Vigna radiata* L.) in Karnataka, India, *Int.J.Microbiol. App.Sci.* 6: 844-852.
- Dutta, G.R., Roy, A.K. 1987.Mycoflora associated with *Strychnos* seeds and deterioration of their active principles under storage. *Ind. Phytopath.* **40**: 520- 524.
- Funder, S. 1968. Practical Mycology: Manual for identification of Fungi. Oslo, Norway, Broggers Boktr. A/S publisher, 144pp.
- Gilman, J.C. 1957. A manual of soil fungi. 2nd ed. Iowa State Univ. Press. USA, pp.450. ISTA, 1976. International Rules for Seed Testing. *Seed Sci. Technol.* 4: 51- 17.
- Jalander, V.,Gachande, B. D. 2012. Effect of fungal metabolites of some rhizosphere soil fungi on seed germination and seedling growth of some pulses and cereals.*Sci. Res. Report.* **2**: 265-267
- Kandhare A. S. 2020. The Common Mycoflora in Four Legumes Seeds and their Effects on Seedling Vigor index. *Middle East* J. Agricul. Res. 9:215-219. DOI: 10.36632/ mejar/2020.9.1.18.
- Kushwaha, A. K. 2021. Seed mycoflora of mung bean and their role in plant health. J. Mycopathol. Res. **59**: 311-314.
- Muskett, A.E. 1948. Techniques for the examination of seeds for the presence of seed-borne fungi. *Trans. Brit. Mycol. Soc.*30: 74-83.
- Rettinassababady, C.,Ramadoss, N.,Ramanadane, T. 2000. Effects of culture filtrates of *Trichoderma viride* isolates on germination of black gram and scleortia of *Macrophomina phaseolina*. *Seed Res.***28**: 193-195.
- Sitara, U., Hasan, N. 2011. Studies on the efficacy of chemical and non- chemical treatments to control mycoflora associated with Chilli seed. *Pak. J. Bot.* **43**: 95-110.
- Srivastava, A.K., Sareen, K. 1972. Germination of soybean seeds as affected by different storage conditions. *Bull. Grain Technol.* **10**: 190-193.
- Than, P.P., Prihastuti, H., Phoulivong, S., Taylor, P.W.J., Hyde, K.D. 2008. Chilli anthracnose disease caused by *Collectotrichum* species. J. Zhejiang. Uni. Sci. 9: 764-778.
- Vijayan, A.K., Rehill, P.S. 1990. Effect of culture filtrates of some seed- borne fungi of *Dalbergia sissoo Roxb*. on seed germination and seedling growth. *Ind. Forester.* **116**: 559-563.